GR

Orginial Submission

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INEOS Fluor

INEOS Fluor Limited
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Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St SW
Washington, DC 20204

Our Ref.	Direct Line	Ext	Date
fgcr48a1	01928 515081	5081	23 August 2001

Dear Sir/Madam

Subject: Notice of GRAS exemption for 1,1,1,2-tetrafluoroethane (HFC-134a)

Pursuant to the proposed rule outline at 62 Fed. Reg. 18939 (April 17, 1997) INEOS Fluor Ltd. hereby submits notification that the use of 1,1,1,2-tetrafluoroethane (HFC-134a) as an extraction solvent in the production of food flavors and flavorings is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

To facilitate your review, this notification is submitted in triplicate in the format suggested under proposed 21 C.F.R. § 170.36(c), see 62 Fed. Reg. at 18961. Also enclosed is an electronic copy (Microsoft Word 97) of the GRAS Exemption Claim (GRAS Exemption Claim for HFC-134a.doc) and Additional Information (GRAS Additional Information for HFC-134a.doc) documents.

Sincerely

Dr Gareth C Robinson
Specialty Products Regulatory Manager
INEOS Fluor Ltd.

GRAS Exemption Claim

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GRAS EXEMPTION CLAIM

INEOS Fluor Ltd. hereby claim that the use of 1,1,1,2-tetrafluoroethane (HFC-134a) as an extraction solvent in the production of flavors and flavorings for foods is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because we have determined that such use of HFC-134a is generally recognized as safe (GRAS).

(1) Name and address of the notifier:

Gareth C Robinson, D. Phil,
Speciality Products Regulatory Manager
INEOS Fluor Ltd.
Runcorn Technical Centre
P.O. Box 13
The Heath, Runcorn
Cheshire WA7 4QF
United Kingdom

011-44-1928-518-022
011-44-1928-580-541

(2) Common or usual name of the substance that is the subject of the GRAS exemption claim:

1,1,1,2-Tetrafluoroethane, HFC-134a

(3) Applicable conditions of use of the notified substance:

(a) Foods in which the substance is to be used:

Food flavors and flavorings.

(b) Levels of use in such foods:

Maximum specification residue in the food flavor extract is 1000 ppm.

(c) Purposes for which the substance is used:

Extraction solvent for food flavors and flavorings.

(d) Description of the population expected to consume the substance:

Members of the population who consume foods containing flavors or flavorings.

(4) Basis for the GRAS determination

The basis of the GRAS determination is scientific procedures.

(5) Review and Copying Statement

The data and information that are the basis for INEOS Fluor Ltd.'s GRAS determination are available for the Food and Drug Administration's (FDA's) review and copying at reasonable times at the offices of the notifier, or will be sent to FDA upon request.

23rd August 2001

Dr Gareth C Robinson

Please address correspondence to:

Diane B McColl
Hyman Phelps and McNamara, P.C.
700 Thirteenth Street, N.W.
Suite 1200
Washington, D.C. 20005

Telephone: 202 737 4291

Additional Information

ADDITIONAL INFORMATION

(1) Identity Of The Notified Substance

(a) Chemical Name

1,1,1,2-tetrafluoroethane (HFC-134a)

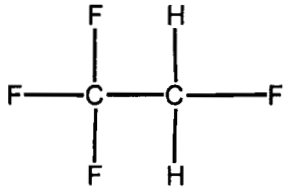
(b) Chemical Abstracts Service (CAS) Registry Number

811-97-2

(c) Empirical Formula

$C_2F_4H_2$

(d) Structural Formula



(e) Method of Manufacture

Industrial grade, or crude 1,1,1,2-tetrafluoroethane (HFC-134a) is produced by the reaction of trichloroethylene with hydrogen fluoride in the presence of chromium-based catalysts. The manufacturing process is a gas phase reaction with 2-chloro-1,1,1-trifluoroethane as an intermediate and hydrogen chloride as the major by-product. In the production of HFC-134a for use as a flavor extraction solvent, crude 1,1,1,2-tetrafluoroethane, which must comply with the appropriate specification, is fed into a two-stage process where it undergoes further purification in discrete batches. The composition of the batch is confirmed by analysis and if satisfactory, the batch is transferred to storage tanks.

(f) Characteristic Properties

At room temperature and pressure, HFC-134a is a colorless gas with a faint ethereal odor.

(g) Any Content of Potential Human Toxicants

None.

(h) Specifications for Food-Grade Material

There are no intermediates in the process for the manufacture of HFC-134a intended for use as an extraction solvent in the production of flavors and flavorings. The specification for HFC-134a contains tests for appearance, identity by gas chromatography (GC) and infrared spectroscopy (IR), water content, acidity, high boiling matter, non-condensable gases, content of organic impurities, and purity. Product specifications are presented in Appendix A. This range of tests is the same as those applied to HFC-134a used in medical applications and is similarly considered to ensure satisfactory quality of the material for use as an extraction solvent in the production of flavors and flavorings. The specification for HFC-134a includes a test for impurities by GC. The named compounds in the specification are considered to be potential impurities in the synthesis of HFC-134a. Limits are set for these impurities which are routinely, or potentially found in the output from the manufacture of HFC-134a. Hydrogen chloride, hydrogen fluoride and water are potential impurities which may be observed in HFC-134a. The content of hydrogen chloride and hydrogen fluoride is controlled by a limit of not more than 0.1 ppm applied to acidity. The limit of not more than 10 ppm w/w for water content has been set based on experience with the manufacture and handling of production scale batches of HFC-134a.

(2) Information On Any Self-Limiting Levels Of Use

None.

(3) Detailed Summary Of The Basis For The Notifier's Determination That A Particular Use Of The Notified Substance is Exempt From The Premarket Approval Requirements Of The Federal Food, Drug, And Cosmetic Act Because Such Use Is GRAS

Based on a critical review and evaluation of the scientific evidence, including e.g., a comprehensive package of publicly available scientific information and data compiled from literature and other published sources (including comprehensive reviews of the safety of HFC-134a), as well as unpublished corroborating data provided by Ineos Fluor, Ltd., and additional data and information concerning the method of manufacture, the chemical and physical properties of the product, the product specifications and analytical data, and the conditions of intended use in production of food flavors and flavorings, independent experts qualified by scientific training and national and international experience concluded that Ineos Fluor Ltd.'s HFC-134a, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is "generally recognized as safe" ("GRAS") based on scientific procedures. A summary of the basis for the experts' determination of GRAS status is provided in the

enclosed "Qualified Experts' Consensus Statement" prepared by Ian C. Munro, Ph.D., FRCPath and Joseph F. Borzelleca, Ph.D.

The Scientific Committee on Food (SCF) of the European Commission accepted the suitability of HFC-134a for use as a solvent for flavor extraction on December 14, 1995 (SCF, 1997). The Solvents Directive (88/344/EEC) was subsequently amended by the European Parliament and by the Council (97/60/EC). Additionally, in the United States, European Union, Canada, Japan and other developed countries, HFC-134a is accepted for use in pharmaceutical applications, as a propellant in metered dose inhalers (MDI) (U.S. FDA, 1998).

(4) Probable Consumption of the Substance

HFC-134a is intended to be used as an extraction solvent for the production of a variety of flavors used in foods. Flavor extracts are prepared from plant materials which are continually washed with liquid HFC-134a, under pressure at ambient temperature, for a length of time suitable for extracting the particular flavor or fragrance. The extract-containing solution is isolated and the HFC-134a solvent is evaporated, condensed and recycled for subsequent use, by compression and condensation. The initial flavor extract can contain residual HFC-134a at levels of a few thousand ppm (w/w). A residual HFC-134a concentration of 1,000 ppm (w/w) has been established as a specification limit in the resultant flavor extracts. Residual HFC-134a levels can be reduced to within specification limits by further evaporation of the extract.

Under the assumption that foods contain 0.1 to 1.0% flavor (FEMA, personal communication) and the adult daily diet is 3,000 g (U.S. FDA, 1999), the consumption of flavors are in the range of 3-30 g per day. Assuming all flavors consumed are resultant from extraction with HFC-134a and all contain the maximum specification residue of 1,000 ppm, the daily intake of HFC-134a is estimated to be 3-30 mg/person/day. Using an adult reference weight of 60 kg, the body weight dose of HFC-134a is estimated to be 0.05-0.5 mg/kg body weight/day. Based on these assumptions, this intake estimate is not only conservative, but likely overestimates exposure, since not all flavors used in foods will contain residual HFC-134a, 1,000 ppm represents the maximum residual HFC-134a, and not all diets will include foods containing HFC-134a extracted flavors.

For comparison, metered dose inhalers (MDIs) deliver 75 mg HFC-134a per inhalation dose (Ventresca, 1995; Alexander *et al.,* 1997). On a body weight basis using a 60 kg adult as reference, this is equivalent to 1.25 mg HFC-134a/kg body weight/inhalation. Similarly, Harrison *et al.* (1996) used a 63 µl inhaler valve volume and described the availability of 25 µl valves. Assuming an HFC-134a liquid density of 1.207 g/ml, the dose of HFC-134a delivered per inhalation is approximately 76 or 30 mg with each of these valve volumes. On a body weight basis using a 60 kg adult as reference, this is equivalent to 1.25 or 0.5 mg HFC-134a/kg body weight/inhalation. It is not atypical for

individuals using metered dose inhalers to require multiple inhalations per day, hence increasing their daily dose of HFC-134a (Ventresca, 1995). HFC-134a has been approved for such use and exposures are considered safe. Thus, assuming MDI users to represent the population of greatest exposure to HFC-134a, the consumption of the highest residual concentration of HFC-134a in food, over a daily 24 hour period, would represent only 40% of the acute exposure of a single 75 mg inhalation from an MDI device.

(5) Basis For Concluding, In Light Of The Data And Information Described Above, That There Is Consensus Among Experts Qualified By Scientific Training And Experience To Evaluate The Safety Of Substances Added To Food That There Is Reasonable Certainty That The Substance Is Not Harmful Under The Intended Conditions Of Use

See the enclosed "Qualified Experts' Consensus Statement: The Generally Recognized as Safe (GRAS) Status of HFC-134a" by Ian C. Munro, Ph.D., FRCPath and Joseph F. Borzelleca, Ph.D.

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Appendix A

Product specifications of HFC-134a intended for use as an extraction solvent in the production of food flavors and flavorings.

	Specification Limits
Appearance	Colorless highly volatile liquid
Water content (ppm, w/w)	≤10
Acidity (ppm, as hydrogen chloride)	≤0.1
High boiling matter (%, v/v)	≤0.01
Identity	
by GC	The principal peak in the GC chromatogram corresponds with the peak produced by the authentic sample
by IR	Concordant with the spectrum of authentic 1,1,1,2 – Tetrafluoroethane
Impurities by GC (ppm, w/w)	
Total Impurity Level	≤ 1000
provided that within the total:	
2-Chloro-1,1,1,2-tetrafluorethane (124)	≤90
2-Chloro-1,1,1-trifluoroethane (133a)	≤3
1,1-Dichloro-1,2,2,2-tetrafluoroethane (114a)	≤10
1,2-Dichloro-1,2,2,2-tetrafluoroethane (114)	≤15
1,1,2,2-Tetrafluoroethane (134)	≤800
1,1,1-Trifluoroethane (143a)	≤80
Any other identified saturated impurity	≤5 (each)
Total unsaturated impurities	≤5
Purity by GC (%, w/w)	99.9-100.0

Qualified Experts'
Consensus Statement

QUALIFIED EXPERTS' CONSENSUS STATEMENT: THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF HFC-134a AS AN EXTRACTION SOLVENT IN FOOD FLAVORS & FLAVORINGS

As independent scientific experts, qualified by training and relevant national and international experience to evaluate the safety of food and food ingredients, we have reviewed the HFC-134a (1,1,1,2-tetrafluoroethane)[1] product intended for use as an extraction solvent in the production of flavors and flavorings for foods, at the request of Ineos Fluor Ltd. ("Ineos Fluor").

We critically evaluated a comprehensive package of publicly available scientific information and data compiled from literature and other published sources, including comprehensive reviews of the safety of HFC-134a, as well as unpublished corroborating data and information. In addition, we evaluated data and information, provided by Ineos Fluor, concerning the method of manufacture, the chemical and physical properties of the product, the product specifications and analytical data, and the conditions of intended use in the production of food flavors and flavorings.

Following independent, critical evaluation of such data and information, and other pertinent information deemed necessary, we conclude that Ineos Fluor's HFC-134a product, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is "generally recognized as safe" ("GRAS") based on scientific procedures under the conditions of intended use as an extraction solvent in the production of food flavors and flavorings. A summary of the basis for our conclusion is provided below.

Chemistry and Manufacturing Process

At room temperature and pressure, HFC-134a (CAS No. 811-97-2) is a colorless gas with a faint ethereal odor. Industrial grade, or crude 1,1,1,2-tetrafluoroethane (HFC-134a) is produced by the reaction of trichloroethylene with hydrogen fluoride in the presence of chromium-based catalysts. The manufacturing process is a gas phase reaction with 2-chloro-1,1,1-trifluoroethane as an intermediate and hydrogen chloride as the major by-product. In the production of HFC-134a for use as a flavor extraction solvent, crude 1,1,1,2-tetrafluoroethane, which must comply with the appropriate specifications, is fed into a two-stage process where it undergoes further purification in discrete batches. The composition of the batch is confirmed by analysis and if satisfactory, the batch is transferred to storage tanks.

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1 Commonly used names for 1,1,1,2-tetrafluoroethane include HFC-134a and HFA-134a. For purposes of this report, we will use HFC-134a throughout (unless we quote an author that used a synonym).

Specifications for Food-Grade Material

There are no intermediates in the process for the manufacture of HFC-134a intended for use as an extraction solvent in the production of flavors and flavorings. The specification for HFC-134a contains tests for appearance, identity by gas chromatography (GC) and infrared spectroscopy (IR), water content, acidity, high boiling matter, non-condensable gases, content of organic impurities, and purity. Product specifications are presented in Appendix A. This range of tests is the same as those applied to HFC-134a used in medical applications and is similarly considered to ensure satisfactory quality of the material for use as an extraction solvent in the production of flavors and flavorings. The specification for HFC-134a includes a test for impurities by GC. The named compounds in the specification are considered to be potential impurities in the synthesis of HFC-134a. Limits are set for these impurities which are routinely, or potentially found in the output from the manufacture of HFC-134a. Hydrogen chloride, hydrogen fluoride and water are potential impurities which may be observed in HFC-134a. The content of hydrogen chloride and hydrogen fluoride is controlled by a limit of not more than 0.1 ppm applied to acidity. The limit of not more than 10 ppm w/w for water content has been set based on experience with the manufacture and handling of production scale batches of HFC-134a.

Intended Use

HFC-134a is intended to be used as an extraction solvent for the production of a variety of flavors used in foods. Flavor extracts are prepared from plant materials which are continually washed with liquid HFC-134a, under pressure at ambient temperature, for a length of time suitable for extracting the particular flavor or fragrance. The extract-containing solution is isolated and the HFC-134a solvent is evaporated, condensed and recycled for subsequent use, by compression and condensation. The initial flavor extract can contain residual HFC-134a at levels of a few thousand ppm (w/w). A residual HFC-134a concentration of 1,000 ppm (w/w) has been established as a specification limit in the resultant flavor extracts. Residual HFC-134a levels can be reduced to within specification limits by further evaporation of the extract.

Under the assumption that foods contain 0.1 to 1.0% flavor (FEMA, personal communication) and the adult daily diet is 3,000 g (U.S. FDA, 1999), the consumption of flavors are in the range of 3-30 g per day. Assuming all flavors consumed are resultant from extraction with HFC-134a and all contain the maximum specification residue of 1,000 ppm, the daily intake of HFC-134a is estimated to be 3-30 mg/person/day. Using an adult reference weight of 60 kg, the body weight dose of HFC-134a is estimated to be 0.05-0.5 mg/kg body weight/day. Based on these assumptions, this intake estimate is not only conservative, but likely overestimates exposure, since not all flavors used in foods will contain residual HFC-134a, 1,000 ppm represents the maximum residual HFC-134a, and not all diets will include foods containing HFC-134a extracted flavors.

For comparison, metered dose inhalers (MDIs) deliver 75 mg HFC-134a per inhalation dose (Ventresca, 1995; Alexander *et al.*, 1997). On a body weight basis using a 60 kg adult as reference, this is equivalent to 1.25 mg HFC-134a/kg body weight/inhalation. Similarly, Harrison *et al.* (1996) used a 63 µl inhaler valve volume and described the availability of 25 µl valves. Assuming an HFC-134a liquid density of 1.207 g/ml, the dose of HFC-134a delivered per inhalation is approximately 76 or 30 mg with each of these valve volumes. On a body weight basis using a 60 kg adult as reference, this is equivalent to 1.25 or 0.5 mg HFC-134a/kg body weight/inhalation. It is not atypical for individuals using metered dose inhalers to require multiple inhalations per day, hence increasing their daily dose of HFC-134a (Ventresca, 1995). HFC-134a has been approved for such use and such exposures are considered safe. Thus, assuming MDI users to represent the population of greatest exposure to HFC-134a, the consumption of the highest residual concentration of HFC-134a in food, over a daily 24 hour period, would represent only 40% of the acute exposure of a single 75 mg inhalation from a MDI device.

Current Regulatory Status

The Scientific Committee on Food (SCF) of the European Commission accepted the suitability of HFC-134a for use as a solvent for flavor extraction on December 14, 1995 (SCF, 1997). The Solvents Directive (88/344/EEC) was subsequently amended by the European Parliament and by the Council (97/60/EC). Additionally, in the United States, European Union, Canada, Japan, and other developed countries, HFC-134a is accepted for use as a propellant in metered dose inhalers (MDIs) (U.S. FDA, 1998).

Safety Database

To obtain the necessary information, comprehensive searches of the published scientific literature were conducted covering the period from 1966 to Feb. 2000. Medline and Toxline served as the primary source of published literature on the safety of HFC-134a, with additional study reports provided by the supplier. Although most studies conducted in animals and humans have been through inhalation routes of exposure, the safety of ingested HFC-134a can be inferred from the toxicological data compiled.

Absorption, Distribution, Metabolism, and Excretion

Absorption, distribution, metabolism, and excretion studies of HFC-134a in animals and humans are limited to inhalation exposures; however, studies indicate poor absorption, rapid equilibration, minimal metabolism, and rapid excretion. In rats exposed to ^{14}C-labelled HFC-134a for 1 hour, absorption of HFC-134a from the respiratory tract was poor, with the sum of radioactivity in the expired air, urine, and feces comprising only 1% of the inhaled dose (Ellis *et al.*, 1993). Of this 1%, approximately two-thirds was exhaled within 1 hour as unchanged HFC-

134a. The remaining radioactivity was excreted within 24 hours of exposure primarily as carbon dioxide. Lesser amounts of radioactivity were recovered in the urine and feces. The only radiolabelled metabolite identified in the urine and feces was trifluoroacetic acid. Tissue analyses 5 days post exposure indicated a uniform distribution of radioactivity without accumulation in specific organs or fat. In humans, the administration of 20 μCi of ^{18}F-HFC-134a in 75 mg HFC-134a, as one inhalation dose, to 7 healthy males and 4 males with severe chronic obstructive pulmonary disease (COPD) demonstrated rapid elimination through ventilation for all subjects, with an apparent initial phase half-life of <5 minutes (Ventresca, 1995). At 10 minutes after inhalation, only approximately 10% of radioactivity was retained. Ventresca (1995) concluded that "in both healthy subjects and severe COPD patients HFA 134a is rapidly eliminated by exhalation, with no accumulation in any region of the body, without significant metabolism, and with no accumulation on repeat dosing."

Cytochrome P-450 IIE1-catalyzed oxidative defluorination is the predominant metabolic pathway for HFC-134a in human, rabbit, and rat liver microsomes *in vitro*, resulting in inorganic fluoride and trifluoroacetic acid (Olsen *et al.*,1990a,b; Olsen *et al.*, 1991; Olsen and Surbrook, 1991; Surbrook and Olsen, 1992). Ellis *et al.* (1993) confirmed an oxidative metabolic pathway for HFC-134a *in vivo,* with metabolism to carbon dioxide and trifluoroacetic acid reported in exposed rats. Trifluoroacetylated proteins were not detected in rats exposed to an atmospheric concentration of 10,000 ppm (42,500 mg/m^3) HFC-134a for 6 hours, indicating that metabolism did not form radicals or other reactive intermediates, such as a trifluoroacetyl halide (Harris *et al.*, 1992).

Inhaled HFC-134a is poorly absorbed, with arterial blood concentrations of HFC-134a rapidly equilibrated and linearly correlated with atmospheric exposure concentrations. In rats, Riley *et al.* (1979) reported the blood concentration of HFC-134a represented 0.129% the exposure concentration. In rats exposed to 2,500, 10,000 or 50,000 ppm HFC-134a by snout-only inhalation for 1 hour/day throughout gametogenesis, mating, pregnancy and lactation, Alexander *et al.* (1996) reported a rapid elimination of HFC-134a without apparent accumulation on repeat dosing. In male rats, Alexander *et al.* (1996) reported a mean half-life of 5.8 minutes, with mean maximum blood concentrations of HFC-134a ranging from 2.9 to 68.2 mg/l during exposure on week 15 of the study. Similar observations were reported with females exposed to 1,800, 9,900, or 64,400 ppm HFC-134a for 1 hour/day on days 17 to 20 of pregnancy and days 1 to 21 post partum. Alexander *et al.* (1996) reported a mean half-life of 7 minutes, with mean maximum blood concentrations ranging from 1.3 to 69.0 mg/l during exposure on day 17 of pregnancy, to 3.5 to 84.7 mg/l during second week post partum exposure. In male and female rats exposed to an atmospheric concentration of 150,000 ppm HFC-134a through head-only inhalation for 60 minutes, a steady state *in vivo* concentration of HFC-134a of approximately 240 mg/kg body weight was obtained after 25 minutes of exposure (Finch *et al.*,1995). Upon termination of exposure, HFC-134a was eliminated rapidly, without detected metabolites, following first-order kinetics with an estimated half-life of approximately 5 minutes in both male and female rats. In adult human males exposed to approximately 75 mg. HFC-134a per inhalation as a propellant in

a 28-day MDI study, Harrison *et al.* (1995) reported blood concentrations of HFC-134a ranging from 0.3 to 1.2 mg/l (4 inhalations, 4 times per day) and from 0.6 to 2.4 mg/l (8 inhalations, 4 times per day), one minute following final exposure. Approximately 15 minutes post exposure, blood concentrations of HFC-134a were reduced by >90% in both dosing procedures, and at 2 hours post exposure, no HFC-134a was detected. Similarly, Donnell *et al.* (1995) reported a median HFC-134a blood concentration of approximately 0.5 mg/l, immediately following a cumulative dose exposure of 16 MDI inhalations (75 mg/inhalation) in 12 healthy adult males. In healthy males exposed to a maximum of 10 inhalations, 75 mg HFC-134a/inhalation, 4 times per day for 2 weeks, peak blood concentrations were variable and dose dependent, although in the range of 1.2-1.4 mg HFC-134a/l within 30-60 seconds of single dose exposure (Ventresca, 1995). In 8 healthy adults exposed to whole body atmospheric concentrations of 1,000, 2,000, 4,000 and 8,000 ppm HFC-134a, Emmen *et al.* (2000) reported a rapid absorption and rapid elimination of HFC-134a in both males and females. Blood concentrations of HFC-134a increased rapidly and were near maximum and equilibrium after 15 minutes of exposure. Maximum blood concentrations were dose dependent, with means of 1.0, 1.9, 3.8 and 7.2 mg/l reported in males at exposure concentrations of 1,000, 2,000, 4,000 and 8,000 ppm HFC-134a, respectively. Respective mean maximum blood concentrations were slightly lower in females. Following the exposure period, Emmen *et al.* (2000) reported the half-life of elimination to be independent of gender and exposure concentration, and biphasic in most subjects, with a mean initial phase half-life of 9 minutes. At 1 hour following exposure to 8,000 ppm HFC-134a, mean blood concentration was <1 mg/l, and only one individual had a detectable level of HFC-134a at 24 hours following exposure.

Mutagenicity/Genotoxicity

The compilation of numerous *in vitro* and *in vivo* assays conducted with HFC-134a indicate an absence of mutagenic and genotoxic potential (Brusick, 1976; Anderson and Richardson, 1979; Hodge *et al.*, 1979; Longstaff *et al.*, 1984; Callander and Priestley, 1990; Mackay, 1990; Trueman, 1990; Collins *et al.*, 1995) (Appendix B).

Acute and Sub-chronic Toxicology Studies

An oral LD50 for HFC-134a is not available and an oral reference dose has not been calculated (EPA, 1999); however, it is of low acute toxicity by the inhalation route (Alexander, 1995). A summary of the results of safety evaluation studies of HFC-134a generated by the Programme for Alternative Fluorocarbon Toxicity Testing, reports an absence of acute toxicity in rats and mice exposed to concentrations of 810,000 ppm HFC-134a for 1 hour (Alexander, 1995). Kennedy (1979a) exposed groups of six male albino rats to 4 hour mean atmospheric concentrations of up to 652,700 ppm HFC-134a, with no mortality observed at concentrations <566,700 ppm. Shulman and Sadove (1967) reported HFC-134a was not lethal to dogs exposed to inhalation concentrations of 700,000 ppm and 800,000 ppm for 3 to 5 hours.

Exposure to HFC-134a did not have a significant effect on body weight gain, haematology, blood chemistry, respective organ weight, or respective organ pathology of rats exposed to a single concentration of 100,000 ppm HFC-134a for 6 hours/day, 5 days/week, in a 14-day inhalation study (Kennedy, 1979b). A significantly higher fluoride content in the urine of exposed rats, taken following the ninth exposure, was indicative of HFC-134a metabolism. The fluoride content in the urine of exposed rats was similar to that of controls following a 14 day recovery period. A GLP-compliant[2] inhalation study with rats exposed to 0, 1,000, 10,000, or 50,000 ppm HFC-134a for 6 hours/day for 20 days in a 28 day period did not produce abnormalities regarding total body weight, clinical signs, food intake and utilization, haematology, blood chemistry, urine composition, and ophthalmoscopy; however, increased absolute liver and kidney weights, and reduced gonad weight, were observed in male rats exposed to 50,000 ppm (Riley *et al.*, 1979). Liver weights were also increased in male rats exposed to 10,000 ppm HFC-134a. Pathological changes were not observed in these tissues and these results were interpreted as physiological adaptations to treatment, as compared to effects of toxicological significance. Male rats exposed to 50,000 ppm HFC-134a also exhibited a greater incidence of mild interstitial pneumonia, manifested as slight focal lesions, that was of toxicological significance related to HFC-134a exposure. These effects; however, have not been observed in subsequent studies. Thus, the NOAEL was 1,000 ppm HFC-134a; however, Riley *et al.* (1979) suggested a toxicological no adverse effect exposure concentration approaching 10,000 ppm. Using a NOAEL of 1,000 ppm HFC-134a (4,250 mg/m^3) (SCF, 1997), this was equivalent to a dose of approximately 1,860 mg/kg body weight/day, based on an average male rat weight of 137 g exposed for 6 hours/day, with an inhalation rate of 0.06 m^3/6 hours (EPA, 1988).

A 90-day GLP-compliant inhalation study (Hext,1989; Collins *et al.*, 1995) using rats exposed to atmospheric concentrations of 0, 2,000, 10,000, or 49,500 ppm HFC-134a for 6 hours/day, 5 days/week, for 13 weeks, reported no toxicity or HFC-134-related effects at any dose level. Significant differences observed in some measured parameters of urine and blood, were not consistently related to dose or duration of exposure to HFC-134a (Hext, 1989). Urinary fluoride concentrations were not significantly elevated in HFC-134a-exposed rats, indicating limited metabolism (Hext, 1989). The exposure of rats to HFC-134a for 13 weeks, was without reported macroscopic or microscopic pathological effects on any organ or tissue (Hext, 1989; Collins *et al.*, 1995). Hext (1989) concluded that "the no effect level can therefore be considered to be in excess of 49500 ppm (v/v) HFC-134a." The highest exposure concentration of 49,500 ppm HFC-134a (212,000 mg/m^3) (SCF, 1997) used in this study, was equivalent to doses of approximately 54,360 and 58,620 mg/kg body weight/day for female and male rats, respectively, based on average female and male rat weights of 156 and 217 g exposed for 6 hours/day, with respective inhalation rates of 0.04 and 0.06 m^3/6 hours (EPA, 1988). On the basis of minor hematological and biochemical effects observed at concentrations ≥10,000 ppm HFC-134a, a conservative no effect level of 2,000 ppm was set in the opinion of the European Scientific

2 The term "GLP-compliant" is cited when reported as such by the author.

Committee on Food (SCF, 1997). However, the SCF stated that " the toxicological significance of the findings was doubtful" (SCF, 1997). An exposure of 2,000 ppm would be equivalent to doses of approximately 2,180 and 2,350 mg/kg body weight/day for female and male rats, respectively, using the aforementioned conditions of exposure.

Cardiac sensitization, reported as the presence of multiple multifocal ectopic beats following administration of adrenaline, has been reported in dogs during high dose inhalation exposure to HFC-134a. Mullin (1979) concluded HFC-134a to have weak cardiac sensitization potential with effects at concentrations ≥ 75,000 ppm and Hardy *et al.* (1991) concluded that an air concentration of between 160,000 and 320,000 ppm HFC-134a was required to cause cardiac sensitization in 50% of exposures. Cardiac sensitization was only observed in dogs with a blood concentration of ≥55 mg/l; however, some dogs had no response to HFC-134a at blood concentrations up to 86 mg/l (Hardy *et al.*, 1991). In comparison, the highest human blood concentration reported with individuals dosed with eight inhalations of HFC-134a through a MDI was approximately 2.4 mg/l (Harrison *et al.*, 1995).

Chronic Toxicity Studies

Longstaff *et al.* (1984) conducted the only reported oral toxicity assay related to HFC-134a, in which male and female rats were treated with 300 mg HFC-134a/kg body weight/day in corn oil by gavage, 5 days/week, for 52 weeks. Following the 52 week treatment period, rats were maintained until the study was terminated at 125 weeks. The treatment of rats with HFC-134a did not increase the incidence of tumors in any organ as compared to the corn oil control group. Although this study is limited in its interpretation to only one dose level and that the extent of absorption was not determined, Longstaff *et al.* (1984) stated the 3% (w/v) HFC-134a in corn oil solution used for dosing approached the maximum solubility based on the volatility of these compounds. This concentration greatly exceeds the intended maximum residual concentration of 1000 ppm HFC-134a (0.1%) in extracted flavors, and even further exceeds the concentration of HFC-134a that may occur in the food products in which these flavors are incorporated.

A chronic GLP-compliant 2-year toxicity and carcinogenicity study was conducted in Wistar-derived rats exposed through inhalation to atmospheric concentrations of 0, 2,500, 10,000 or 50,000 ppm HFC-134a, 6 hours/day, 5 days/week (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995). In animals evaluated at 52 weeks, there was no evidence of toxicity or HFC-134a-related effects on any physiological parameter measured. Over the 2 year duration of study, there were no treatment-related effects on body weight, body weight gain, food consumption, or in clinical condition of both sexes at all concentrations of HFC-134a. Complete post-mortem and histopathological examination of all rats at the conclusion of exposure revealed a statistically significant higher mean relative testes weight, which correlated with an increased incidence of Leydig cell hyperplasia and benign Leydig cell tumors, in male rats exposed to 50,000 ppm HFC-134a. This observation was the only event of toxicological significance in rats related to chronic, high level exposure to HFC-134a. The mechanism of toxicity of HFC-134a to male rat

Leydig cells is not known, but is likely a non-genotoxic effect involving hormonal disruption (Clegg *et al.*, 1997). Considering the lack of genotoxic potential of HFC-134a, and the absence of hyperplasia and tumors at 52 weeks, this treatment-related result was interpreted as age-dependent, occurring through an undetermined non-genotoxic mechanism (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995). Although exposure to 50,000 ppm HFC-134a for 2 years resulted in a significantly greater incidence of Leydig cell hyperplasia than controls (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995), a number of diverse chemicals of various chemical structure also induce this response in rats without apparent similar effect in humans (Griffith, 1988; Bär, 1992; Clegg *et al.*, 1997). Furthermore, Wistar-derived rats exhibit a spontaneous incidence of benign Leydig cell tumors that range to near 100% (Bär, 1992). Hence, the occurrence of increased Leydig cell hyperplasia in high dosed rats is of questionable significance relevant to risk to human health (Bär, 1992; Clegg *et al.*, 1997). Accounting for the inherent uncertainty in extrapolating the incidence of Leydig cell hyperplasia from rats to humans, and the high dose and chronic duration of HFC-134a exposure necessary to produce such an effect in rats, an increased risk of testicular toxicity is not considered relevant in regard to human exposure to minimal residual HFC-134a concentrations in foods. A NOAEL of 10,000 ppm HFC-134a (42,500 mg/m^3) (SCF, 1997) in male rats is equivalent to a dose of approximately 12,085 mg/kg body weight/day, based on an average male rat weight of 211 g exposed for 6 hours/day (Hext and Parr-Dobrzanski, 1993), with an inhalation rate of 0.06 m^3/6 hours (EPA, 1988).

Developmental and Reproductive Toxicology

Hodge *et al.* (1980) conducted a GLP-compliant teratogenicity study in female Sprague-Dawley rats exposed to whole body atmospheric concentrations of 0, 1,000, 10,000 and 50,000 ppm HFC-134a (v/v) for 6 hours/day on days 6 through 15 of gestation. Rats were sacrificed on day 21 of gestation and subject to full pathology. Exposure to HFC-134a had no adverse effect on maternal body weight gain, or the number of fetal or embryonic deaths. No significant effects were reported for the numbers of implantations, live fetuses or resorptions, mean uterus weights, and mean litter weights, associated with exposure to HFC-134a; however, mean fetal weight was slightly, but significantly, reduced at 50,000 ppm suggesting a fetotoxic effect. Maternal exposure to 50,000 ppm HFC-134a resulted in statistically significant retardation of ossification of the vertebrae, sternebrae, digits and calcaneum in the fetus. A study of the embryotoxic and teratogenic effects of 30,000, 100,000 and 300,000 ppm HFC-134a on female Sprague-Dawley rats exposed through inhalation for 6 hours/day from days 6-15 of gestation resulted in a significant reduction in mean feed consumption and weight gain of dams exposed to 300,000 ppm (Lu, 1981). Consistent with Hodge (1980) although at a higher dose, Lu (1981) reported the mean fetal weight of dams exposed to 300,000 ppm was significantly reduced as compared to controls, in the absence of any other adverse reproductive effects associated with exposure to HFC-134a. Furthermore, Lu (1981) also reported that maternal exposure to 300,000 ppm HFC-134a had a significant effect of impaired skeletal ossification of the fetus.

Collins *et al.* (1995) reported a GLP-compliant developmental toxicity study in artificially inseminated, female New Zealand white rabbits exposed to atmospheric concentrations of 0, 2,500, 10,000, or 40,000 ppm HFC-134a for 6 hours/day on days 7 through 19 of pregnancy. The study was terminated on day 30 of gestation, and a full post mortem examination of each dam and fetus was conducted. Slight maternal toxicity was reported at 40,000 ppm HFC-134a, as indicated by a lower rate of body weight gain as compared with control; however, data was not shown and statistical significance was not reported. Exposure to HFC-134a had no adverse clinical effects on female rabbits, and the mean numbers of corpora lutea, implantations, and live fetuses were similar across treatments and to the control. Furthermore, exposure to HFC-134a had no effect on litter weights, pup sex ratios, or the incidence of adverse developmental conditions, as compared with the controls at all exposure concentrations (Collins *et al.*, 1995).

Alexander *et al.* (1996) assessed the reproductive toxicity of HFC-134a in GLP-compliant fertility and peri- and post-natal studies in random bred albino (AHA) strain rats. In the fertility study, groups of male and female rats were exposed by snout-only inhalation to atmospheric concentrations of 0, 2,500 10,000 or 50,000 ppm HFC-134a, for 1 hour/day throughout gametogenesis, mating, pregnancy and lactation. Specifically, males were exposed for 18 continuous weeks, from 10 weeks prior to mating to termination 8 weeks post mating. Females were exposed 3 weeks prior to mating and continued through day 20 of pregnancy, and recommenced on day 1 post partum to termination at day 21 post partum. Selected F_1 offspring were raised to maturity, mated, and the resultant F_2 progeny terminated at sexual maturity. In the peri- and post-natal study, mated female rats were exposed to inhalation concentrations of 0, 1,800, 9,900 or 64,400 ppm HFC-134a for 1 hour/day on days 17 through 20 of pregnancy and days1 through 21 post-partum. Developmental parameters of the F_1 generation were assessed and selected rats mated and terminated on day 20 of pregnancy. Overall, these studies demonstrated HFC-134a had no effect on the reproductive performance and fertility of the rat, or on the *in utero* and post natal development of two successive generations. In both studies, clinical effects or mortalities related to HFC-134a exposure were not observed in F_0, F_1 or F_2 generations. Alexander *et al.* (1996) estimated that exposures at the highest concentration of HFC-134a used in these studies was approximately 4,000-fold greater than the expected human exposure through the clinical use of HFC-134a in such devices as inhalers.

Human Studies

For use as a safe alternative to chlorofluorocarbons (CFC) propellants in MDI applications, HFC-134a has undergone extensive toxicological assessments, which includes safety studies in humans (Alexander, 1995; Ventresca, 1995; Harrison *et al.*, 1996; Ayres *et al.*, 1998). Ventresca (1995) evaluated the safety and tolerability of HFC-134a in healthy and severe chronic obstructive pulmonary disease (COPD) males. Ventresca (1995) evaluated respiratory function, symptoms of irritation of the upper respiratory tract, cardiovascular function, and laboratory parameters, including liver function, in a single ascending dose study and two repeat dose studies in a total of 50 healthy males. Although the dosing protocol was not specified by Ventresca

(1995), subjects were exposed to a maximum of 10 inhalations, 4 times a day for 2 weeks. Each inhalation delivered 75 mg HFC-134a (Ventresca, 1995). Thus, at the maximum dose level, subjects were exposed to 3,000 mg HFC-134a per day for 2 weeks. Ventresca (1995) reported no adverse effects related to exposure to HFC-134a, with no significant changes reported in vital signs, pulmonary function tests, ECG and laboratory parameters. Pharmacokinetics indicated HFC-134a did not accumulate with repeated dosing and did not accumulate in body tissues, with a similar response in healthy and COPD subjects.

To assess the acute safety of inhaled HFC-134a, Donnell *et al.* (1995) conducted a randomized, double-blind, crossover study with 12 healthy adult males, administered cumulative doses of 1, 2, 4, 8 and 16 inhalations on each of three consecutive days. The quantitative dose of HFC-134a per inhalation was not reported by Donnell *et al.* (1995), but was likely similar to the 75 mg reported by Ventresca (1995). Thus, a cumulative exposure of 16 inhalations would equate to 1,200 mg HFC-134a. As compared to baseline measurements, HFC-134a had no effect on the measured parameters of pulmonary function, cardiovascular function, finger tremor, and serum potassium levels.

Harrison *et al.* (1996) conducted a 28-day continuous exposure, double-blind, parallel group study in 16 healthy, non-smoking adult males, to investigate the safety and tolerability of HFC-134a as a MDI propellant, as compared to a reference MDI chlorofluorocarbon propellant. Eight subjects received one of two HFC-134a treatments for 28 days within a 14-day cross-over design. Dosing protocols consisted of 4 inhalations, 4 times per day, or 8 inhalations, 4 times per day, using a 63 µl valve delivering approximately 50 µl HFC-134a per inhalation. Pulmonary function tests were conducted daily, cardiovascular responses were measured on days 1, 7, 8, 14, 15, 21, 22 and 28, and haematology and serum chemistry measured on days 1, 14, 28 and post-study. No adverse effect were observed on heart rate, blood pressure, electrocardiograms, pulmonary function test values, various clinical parameters, or the reported incidence of adverse events in the cardiovascular, respiratory, gastrointestinal, and central nervous systems of dosed subjects. The single exception noted that was considered related to HFC-134a exposure, was one subject with an eosinophil count that increased with the increased duration of study (Harrison *et al.*, 1996). Eosinophil counts of that individual returned to normal range when evaluated six months post-study. As cited by Harrison *et al.* (1996); however, no significant change in eosinophil counts were observed in a 12-week inhaler study with HFC-134a as a propellant (Bleeker *et al.*, 1995). Harrison *et al.* (1996) concluded that "the safety and tolerability of the HFA-134a CFC-free system was demonstrated over 28 days of exposure in healthy subjects."

Vinegar *et al.* (1997) produced a non-peer reviewed report of a study of the pharmacokinetics of inhaled HFC-134a, initiated in 1997 by researchers at the U.S. Air Force Wright-Patterson Medical Center. This study was designed to expose seven healthy adult males to an atmospheric concentration of 4000 ppm (0.4%) HFC-134a for a period of 30 minutes, and to collect physiologically based pharmacokinetic validation data. In addition to HFC-134a, exposures to Halon 1301 and HFC-227ea were also investigated using the same subjects. The exposure

concentration selected for each chemical was considered to be well below any published adverse effect level (Vinegar *et al.*, 1997). Exposure to Halon 1301 was assessed first and was well-tolerated by all subjects, with no reported changes in electrocardiogram (ECG), blood pressure or heart rate. After approximately 4.5 minutes of exposure to HFC-134a, the first subject exposed lost consciousness, and pulse and blood pressure dropped to zero. Treatment was stopped and medical intervention restored pulse and blood pressure. Blood samples through 2.5 minutes of exposure showed a rapid rise in HFC-134a blood concentration to 1.29 mg/l. A second subject terminated exposure at 10.5 minutes, concurrent with a rapid rise in blood pressure and pulse rate. The blood concentration of HFC-134a of this individual was approximately 0.7 mg/l following 10 minutes of exposure. Vinegar *et al.* (1997) suspended study of HFC-134a following these two subjects. Harrison *et al.* (1995) reported blood concentrations of HFC-134a ranging from approximately 0.3 to 2.4 mg/l, in individuals exposed through MDI use. Considering the lack of reported toxicity associated with HFC-134a, Vinegar *et al.* (1997) offered no plausible mechanism for the observed effects. The initial diagnosis of the unconscious subject was vasovagal reflex response; however, the subject had previously completed an inhalation exposure to Halon 1301 without incident, involving multiple blood sampling (Vinegar *et al.*, 1997). The response of the second exposed individual was likely biased by study design, in that exposure was non-blinded and subsequent to treatment of the first subject.

In response to the report of Vinegar *et al.* (1997), double-blind, ascending dose, controlled, GLP-compliant, clinical safety studies on HFC-134a were conducted at the TNO Food and Nutrition Institute in the Netherlands (Emmen *et al.*, 2000). Emmen *et al.* (2000) evaluated the effects of one hour, whole body exposures of 1,000, 2,000, 4,000 and 8,000 ppm HFC-134a on the lung function, blood pressure and pulse rate, ECG, and various blood and serum chemistry parameters in 8 healthy adults (4 males, 4 females). The exposure to HFC-134a was well-tolerated, and no clinical changes or adverse events related to exposure to HFC-134a were reported. In addition to a more rigorous experimental design, the study by Emmen *et al.* (2000) used a higher maximum exposure concentration and longer exposure period than Vinegar *et al.* (1997). Emmen *et al.* (2000) observed HFC-134a to be rapidly absorbed and eliminated in both males and females. Blood concentrations of HFC-134a increased rapidly, approaching maximum following 15 minutes of exposure, and tended to be higher in males than females. At 4,000 ppm HFC-134a, Emmen *et al.* (2000) reported a mean maximum blood concentration of 3.1 and 3.8 mg/l in females and males, respectively. At 8,000 ppm, maximum blood concentrations were 6.0 and 7.2 mg/l in females and males. These blood levels are higher than the values reported by Vinegar *et al.* (1997), and were tolerated without adverse effects. Following the exposure period, Emmen *et al.* (2000) reported the half-life of elimination to be independent of gender and exposure concentration, and biphasic in most subjects, with a mean initial phase half-life of 9 minutes. Only one individual had a detectable level of HFC-134a 24 hours following exposure. The results of Emmen *et al.* (2000) are consistent with the lack of adverse effects reported in animal studies, or observed in the clinical use of HFC-134a. Emmen *et al.* (2000) commented that "the findings reported by Vinegar *et al.* (1997) represent a spurious event unrelated to the inhalation

of . . . HFC 134a . . .." Furthermore, a 3-month post-market surveillance study of 6,614 patients with obstructive airways disease and prescribed MDIs, reported HFC-134a to be as safe as existing chlorofluorocarbon inhalers (Ayres *et al.*, 1998).

In summary, animal toxicity testing with HFC-134a through inhalation exposure at concentrations far in excess of those residual through its intended use in foods, indicate that HFC-134a is of very low toxicity. Numerous *in vitro* and *in vivo* assays confirm the absence of genotoxic potential, and *in vivo* studies demonstrate no developmental toxicity. While oral exposure studies through the consumption of HFC-134a are limited to the results of Longstaff *et al.* (1984), in which an absence of toxicity and tumorogenicity was reported, the minimal metabolism and rapid elimination of HFC-134a in high dose inhalation exposed animals supports a general absence of toxicity. The reported adverse effects of HFC-134a reported by Vinegar *et al.* (1997) in two individuals, is an anomaly in the extensive amount of animal and human data that demonstrate otherwise. Controlled clinical studies (Emmen *et al.*, 2000) did not confirm the observations of Vinegar *et al.* (1997). The clinical testing of HFC-134a as an MDI propellant, without the active drug, for a continuous exposure period of 1 month produced no adverse effects in healthy individuals (Harrison *et al.*, 1996). A post-market surveillance study of patients using inhalers with HFC-134a as a propellant, demonstrated HFC-134a was clinically safe (Ayres *et al.*, 1998). The clinical use of HFC-134a would result in exposures much greater than those estimated from the residual levels of HFC-134a in extracted flavors. An overestimation of human intake of HFC-134a, residual in flavors at a maximum concentration of 1000 ppm, would be in the range of 0.05-0.5 mg/kg body weight/day. This is > 20,000 times less than the inhalation NOAEL in animals (Collins *et al.*, 1995), and > 4,000 times less than the conservative 90-day rat NOAEL reported by the European Scientific Committee on Food (SCF, 1997). Furthermore, the estimated human exposure through maximum occurrence of HFC-134a in foods is approximately 600- to 6000 times less than the oral dose of 300 mg/kg body weight, without effect in rats (Longstaff *et al.*, 1984). Although the absorption of HFC-134a following oral administration has not been determined, the minimal amounts consumed with flavor-containing food, in combination with the high vapor pressure, and minimal metabolism and rapid elimination from the body, indicates residual HFC-134a in flavor extracts, at concentrations up to 1000 ppm, would not be of toxicological concern to human health. On the basis of similar data regarding HFC-134a, the opinion report of the European Scientific Committee for Food concluded that "the use of tetrafluoroethane as a solvent for flavour extraction is regarded as acceptable" (SCF, 1997).

Conclusion

Based on a critical, independent, and collective evaluation of the available data and information, we conclude that Ineos Fluor LTd.'s HFC-134a extraction solvent, meeting the appropriate food grade specifications and manufactured in compliance with current good manufacturing practices, is "generally recognized as safe" ("GRAS") based on scientific procedures under the conditions of intended use in the production of food flavors and flavorings.

Ian C. Munro, Ph.D., FRCPath
Cantox Health Sciences International

August 22, 2001
Date

Joseph F. Borzelleca, Ph.D.
Medical College of Virginia
Virginia Commonwealth University
Toxicology and Pharmacology, Inc.

20 August 2001
Date

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Appendix A. Product specifications of HFC-134a intended for use as an extraction solvent in the production of food flavors and flavorings.

	Specification Limits
Appearance	Colorless highly volatile liquid
Water content (ppm, w/w)	≤10
Acidity (ppm, as hydrogen chloride)	≤0.1
High boiling matter (%, v/v)	≤0.01
Identity	
by GC	The principal peak in the GC chromatogram corresponds with the peak produced by the authentic sample
by IR	Concordant with the spectrum of authentic 1,1,1,2 - Tetrafluoroethane
Impurities by GC (ppm, w/w)	
Total Impurity Level	≤ 1000
provided that within the total:	
2-Chloro-1,1,1,2-tetrafluorethane (124)	≤90
2-Chloro-1,1,1-trifluoroethane (133a)	≤3
1,1-Dichloro-1,2,2,2-tetrafluoroethane (114a)	≤10
1,2-Dichloro-1,2,2,2-tetrafluoroethane (114)	≤15
1,1,2,2-Tetrafluoroethane (134)	≤800
1,1,1-Trifluoroethane (143a)	≤80
Any other identified saturated impurity	≤5 (each)
Total unsaturated impurities	≤5
Purity by GC (%, w/w)	99.9-100.0

Appendix B. Summary of studies evaluating the genetic toxicity of HFA 134a.

Assay	Strain/Cell Type	Assay Conditions	Result	Reference
In Vitro				
Ames: *Salmonella typhimurium*	TA1535, TA1537, TA1538, TA98, TA100	Test conc.: 100%; +/-S9	Negative	Brusick, 1976
Saccharomyces cerevisiae	D4	Test conc.: 100%; +/-S9	Negative	Brusick, 1976
Ames: *Salmonella typhimurium*	TA1535, TA1538, TA98, TA100	Test conc.[a]: ≥50%; +/-S9	Negative	Longstaff *et al.*, 1984
Ames: *Salmonella typhimurium*	TA1535, TA1537, TA1538, TA98, TA100	Test conc.: ≤100%; +/-S9	Negative	Callander and Priestley, 1990
Ames: *Salmonella typhimurium*	TA1535, TA1537, TA98, TA100	Test conc.: 5-100%; +/-S9	Negative	Collins *et al.*, 1995
Escherichia coli	WP2uvrA	Test conc.: 5-100%; +/-S9	Negative	Collins *et al.*, 1995
Styles cell transformation	baby hamster kidney fibroblasts (BHK21)	Test conc.[a]: ≤100%; +S9	Negative	Longstaff *et al.*, 1984
Chromosome aberration	Chinese hamster lung (CHL) cells	Test conc.: 40-100%; +/-S9	Negative	Collins *et al.*, 1995
Chromosome aberration	Human lymphocytes	1 ♂, 1 ♀ donor; Test conc.: ≤750,000 ppm; +/-S9	Negative	Mackay, 1990: Collins *et al.*, 1995
In Vivo				
Dominant lethal	CD1 mice	Inhalation: 1,000, 10,000 or 50,000 ppm; 6 h/d x 5 d; 15 ♂/trt	Negative	Hodge *et al.*, 1979
Micronucleus	NMRKf (SPF71) mice	Inhalation: 50,000 or 150,000 ppm for 6 h, or 500,000 ppm for 5 h; 15 ♂, 15 ♀/trt	Negative	Collins *et al.*, 1995
Chromosome aberration	Alpk/ApfSD (Wistar-derived) rats	Inhalation: 1,000, 10,000 or 50,000 ppm; 6 h/d x 5 d; 8 ♂/trt	Negative	Anderson and Richardson, 1979
Unscheduled DNA synthesis (UDS)	Alpk/ApfSD (Wistar-derived) rats	Inhalation: 50,000 or 100,000 ppm for 6 h; 5 ♂/trt	Negative	Trueman, 1990; Collins *et al.*, 1995

[a] not specified

End Submission

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DIRECT DIAL (202) 737-4291

September 26, 2001

MEMORANDUM

BY FEDERAL EXPRESS

TO: Jason Dietz

FROM: Diane McColl

SUBJECT: GRAS Notice 82

Enclosed is the diskette that should have been enclosed with GRAS Notice 82 for 1,1,1,2-tetrafluoroethane (HFC 134a). I apologize for any inconvenience this oversight may have caused you and your colleagues.

If you have any further questions concerning this GRAS Notice, please do not hesitate to ask.

DBM/dmh
Enclosure

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AM 

Dietz, Jason

From:
Sent: Wednesday, October 03, 2001 3:38 PM
To: Dietz, Jason
Cc: gareth.robinson@ineosfluor.com
Subject: GRAS Notice 82

Dear Jason:

As requested, I have confirmed with Dr. Gareth Robinson at Ineos Fluor, Ltd. that the intended use of 1,1,1,2-tetrafluoroethane (HFC 134a) as an extraction solvent does not include use with animal materials. HFC 134a is intended for use in extracting flavors from a wide range of vegetative materials, including fruits, vegetables and spices, as well as other natural seasonings and flavorings. Hence, the intended use is not limited to the spices and natural seasonings listed in 21 C.F.R. 182.10.

If you have any further questions regarding GRAS Notice 82, please do not hesitate to contact me.

Sincerely,
Diane McColl
Counsel to Ineos Fluor, Ltd.

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
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*NOT ADMITTED IN DC

November 12, 2001

MEMORANDUM

BY FEDERAL EXPRESS

TO: Linda Kahl, Ph.D.
Jason Dietz

COPY: Gareth Robinson Ph D

FROM: Diane McColl

SUBJECT: Ineos Fluor, Ltd. GRAS Notice for 1,1,1,2-tetrafluoroethane (GRN 000082)

As requested, enclosed is a copy of the 1997 opinion by the Scientific Committee For Food (SCF) on the safety of 1,1,1,2-tetrafluoroethane as an extraction solvent for food flavors. Also enclosed for your information is a copy of the 1995 ECETOC Joint Assessment of 1,1,1,2-tetrafluoroethane, which is cited in the SCF opinion.

As to a meeting during the week of November 26, Ineos Fluor's expert consultants and I are available for a meeting at any time on Friday, November 30. Please let me know as soon as possible if this date is convenient for you or if you have any further questions regarding GRAS Notice 000082.

DBM/dmh
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3940.001

000046

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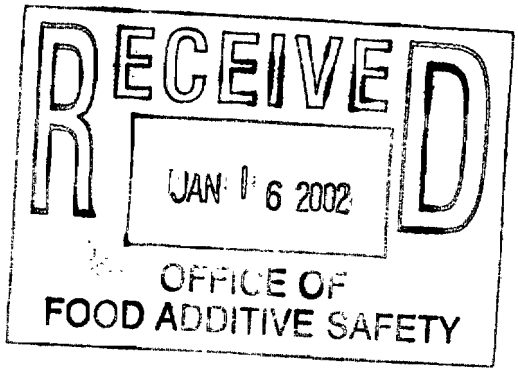
DIRECT DIAL (202) 737-4291

January 14, 2002



BY FEDERAL EXPRESS

Jason Dietz
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College Park, Maryland 20740-3835

Re: Supplement to GRAS Notice (GRN 82) for use of 1,1,1,2-tetrafluoroethane (HFC 134a) as an extraction solvent

Dear Mr. Dietz:

On behalf of Ineos Fluor, Ltd., we submit the enclosed Supplement to the GRAS Notification for HFC 134a (GRN 82). Provided in the Supplement is the requested follow-up discussion by Joseph Borzelleca, Ph.D. and Ian Munro, Ph.D., as members of the Expert Panel that found HFC 134a to be GRAS, joined by John Doull, Ph.D., M.D., concerning:

(1) A refined exposure estimate based on use data for essential oils obtained from the Flavor & Extract Manufacturers' Association's (FEMA's) poundage survey, and analytical data demonstrating HFC 134a residues of < 300 ppm (w/w) in representative extracted flavors;

(2) The basis for reliance on inhalation data to estimate the safety of oral consumption of HFC 134a under the conditions of intended use as an extraction solvent in food flavors production;

000078

(3) The significance of Leydig cell effects observed in the rat inhalation study to human safety of HFC 134a under the conditions of intended use as an extraction solvent; and

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(4) The safety of trace amounts of potential metabolites of HFC 134a under the conditions of intended use.

Three copies of the Supplement are enclosed to facilitate your review. Also included are three copies of the references that are of particular significance to the issues of reliance on inhalation data to establish oral safety (EPA, 1986; Dourson and Felter, 1997), and relevance of rat Leydig cell findings to human safety (Alison *et al.*, 1994; McClain, 1994; Prentice and Meikle, 1995).

As evidenced by the enclosed Supplement and the "Qualified Experts Consensus Statement" provided in the GRAS notice, there exists a consensus among qualified experts that, under the conditions of intended use as an extraction solvent in the production of food flavors, HFC 134a is GRAS based on scientific procedures.

If you have any further questions concerning this matter, please do not hesitate to contact me.

Sincerely,

Diane B. McColl
Counsel to Ineos Fluor, Ltd.

DBM/dmh
Enclosure

SUPPLEMENT TO GRAS NOTIFICATION FOR HFC134a (GRASN 82)

INTRODUCTION

As requested by the U.S. FDA, we as an Expert Panel have provided: a) a refined estimate of human exposure to HFC134a from its intended use as an extraction solvent for food flavoring substances, b) the rationale for the use of inhalation data to assess oral safety, c) a discussion on the potential for HFC134a to induce Leydig cell tumors in light of data on structurally similar compounds, and d) a discussion of the potential for metabolism to fluoroacetate, an inhibitor of the Kreb's cycle. Each of these issues is addressed separately below.

EXPOSURE ESTIMATE

In the original GRAS opinion document, human exposure to HFC134a in foods was conservatively estimated to be in the range of 0.05 to 0.5 mg/kg body weight/day. This estimate was based on the following assumptions: a) a person would consume 3,000 grams of food per day (U.S. FDA, 1999), b) flavoring substances constitute from 0.1 to 1.0% of the diet (*i.e.*, consumption of 3 to 30 grams of flavors/day) (FEMA, personal communication), c) HFC134a would be present in the flavor extracts at the maximum concentration of 1,000 ppm indicated in the product specifications, and d) an adult human reference body weight of 60 kg. The human exposure estimate of 0.05 to 0.5 mg HFC134a/kg body weight/day (*i.e.*, 3-30 grams of flavours/day x 0.001/60 kg) was considered to over-estimate actual human exposure since not all flavors used in foods will contain residual HFC134a, 1,000 ppm represents the maximum residual HFC134a, and not all diets will include foods containing HFC 134a extracted flavors.

To further refine the initial estimates of human exposure of 0.05 to 0.5 mg/kg body weight/day we: a) reassessed daily intake of essential oils, that might be expected to contain HFC134a, based on poundage (use) data, and b) incorporated the results of analytical data showing that HFC134a residues in extracted flavors will not exceed 300 ppm (w/w).

FEMA Poundage Data for Essential Oils

Human exposures to HFC134a, under its intended conditions of use, were estimated based on a Flavor & Extract Manufacturers' Association (FEMA) poundage survey conducted in 1995.

The FEMA survey included data submitted by 63 of its member companies and data from the National Association of Chewing Gum Manufacturers submitted on behalf of its members. More than 2,400 flavoring substances used as food additives were included in the FEMA survey.

The poundage (usage) data on 215 essential oils was extracted from a CD-rom containing the survey results and imported to a spreadsheet. The data were then converted from poundage (pounds manufactured per year) to an estimate of daily *per capita* intake (mg/person/day) using the following calculation:

$$\text{Daily } \textit{per capita} \text{ intake} = \frac{\text{Poundage (lbs/yr)}}{2.2 \text{ lbs/kg} \times 260 \times 10^6 \text{ people} \times 365 \text{ days/yr}} \times 10^6 \text{ mg/kg}$$

The calculation was based on the assumption that a) 100% of the companies producing the essential oil have reported the poundage data; and b) 100% of the U.S. population is consuming the total poundage. The total poundage and estimated daily *per capita* intake of essential oils from use as food additives are presented in Table 1 on a mg and on a mg per kilogram body weight basis.

Table 1 Total Poundage and Daily *Per Capita* Intake of Essential Oils

Total Poundage (lbs/yr)	Intake (mg/person/day)	Intake (mg/kg bw/day)
18,398,552.6	88.12	1.47

The poundage of the essential oils ranged from 0 for a number of oils to 4,650,000 pounds per year for peppermint oil (*Mentha piperita L.*). Additional details of the calculations and of assumptions used are provided in Attachment 1 (report entitled "Estimated Daily Intake of Essential Oils by the U.S. Population from use as Food Additives").

The human intake of essential oils, assuming that consumption was evenly distributed throughout the population and a human body weight of 60 kg, was calculated to be 1.47 mg/kg body weight/day.

Residues of HFC134a in Flavor Extracts

In addition to refining the estimate of the human intake of flavoring substances potentially extracted with HFC134a, the amount of residual solvent remaining in the extracted substances was re-evaluated. In the original report, all flavoring substances were assumed to contain 1,000 ppm HFC134a, the maximum amount indicated in the product specifications. The results of analytical studies conducted by Ineos Fluor, Ltd., shown in Table 2, demonstrate that the actual amount of residual solvent remaining in extracted oils is not likely to exceed 300 ppm (w/w).

Table 2 Residual HFC134a in Extracts Intake of Essential Oils (data from Ineos Fluor, Ltd.)		
Extract	**Solvent Removal Conditions**	**Residual HFC134a (ppm)**
Magnolia bark	nitrogen purge at 45 °C	348
Magnolia bark	nitrogen purge at 60 °C	65
Star anise	nitrogen purge at ambient temperature	not detected
Star anise	evacuation at 50 °C	236
Clove	nitrogen purge at ambient temperature	103
Clove	evacuation at 50 °C	188
Ginger	evacuation at 40 °C	177
Orange	nitrogen purge at ambient temperature	101
Vanilla	nitrogen purge at 50 °C	107
Juniper oil	evacuation at ambient temperature	102

Summary of Refined Human Exposure Estimate

Human exposure to HFC134a under its intended conditions of use is a product of the amount of substances extracted with this solvent and the concentration remaining in the extracted substances. Given the human consumption of products potentially extracted with HFC134a (1.47 mg/kg body weight/day) and the analytical data indicating that HFC134a is not likely to be present in amounts in excess of 300 ppm, human consumption of HFC134a under its intended conditions of use is calculated to be 0.00044 mg/kg body weight/day (*i.e.*, 1.47 mg/kg body weight/day x 300/1,000,000). This value may also over-estimate exposure since HFC134a is highly volatile with a boiling point of –26 °C, and, therefore, would be expected to volatilize from extracts over time. In addition, the estimate assumes that all essential oils are extracted with HFC134a. Processing of food products containing HFC134a extracted flavors would further reduce residual amounts, especially when processing involves heat or vacuum extraction.

The estimated human consumption of 0.00044 mg/kg body weight/day, calculated using poundage data for products likely to be extracted with HFC134a and actual analytical residue

data, as expected, is considerably lower than the crude, but conservative, estimate of human exposure presented in the original report. Compared with the exposure estimate provided in the original report, the refined exposure estimate using essential oil poundage data and measurements of residual HFC134a in extracted products, provides for an additional 113- to 1130-fold margin-of-safety.

USE OF INHALATION DATA TO ESTIMATE ORAL TOXICITY

The results of peer-reviewed inhalation studies (Hodge *et al.*, 1980; Lu, 1981; Hext, 1989; Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995; Alexander *et al.*, 1996) were used as the primary source of data to characterize toxicity potentially associated with trace level oral exposure resulting from its use as an extraction solvent for flavors. These data were supported by a single 52-week oral toxicity study (Longstaff *et al.*, 1984). Details of these studies are provided in the original GRAS notification document.

Since systemic toxicity is related to blood concentrations and area under the curve, inhalation studies that provide for systemic exposure can be used to qualitatively characterize systemic toxicity. In the case of HFC134a, pharmacokinetic studies (Riley *et al.*, 1979; Finch *et al.*, 1995; Alexander *et al.*, 1996) show that inhalation exposure of rats to 10,000 ppm produces blood concentrations of HFC134a in the range of 10 to 20 mg/L (Riley *et al.*, 1979). Given that no systemic toxicity was observed in the chronic studies at 10,000 ppm, it is clear that exposures to HFC134a producing blood concentrations in the range of 10 to 20 mg/L, whether achieved by inhalation or through oral dosing, are not associated with systemic toxicity.

Quantitative estimates of oral toxicity, in the absence of appropriate oral studies, can be established from inhalation data through route-to-route extrapolation that accounts for variances in absorption rates by different routes of exposure (U.S., EPA, 1987; Gerrity and Henry, 1990; Dourson and Felter, 1997). This technique has been used by the U.S. EPA to establish oral reference doses (RfD) on the basis of inhalation studies when no adequate oral studies existed (U.S. EPA, 1987; Dourson and Felter, 1997).

For HFC134a, appropriate pharmacokinetic data exist to establish the extent of absorption by the inhalation route (Ellis *et al.*, 1993). Ellis *et al.* (1993) exposed male and female rats to ^{14}C-labelled HFC 134a at a concentration of 10,000 ppm for a period of one hour. Upon completion of exposure, the rats were removed from the exposure chamber and placed into metabolic cages. Based on the recovery of radioactivity in expired air, urine, and feces following removal from the exposure chamber, Ellis *et al.* (1993) concluded that approximately 1% of the inhaled dose was absorbed. Ellis *et al.* (1993) acknowledged that this underestimated actual absorption since the amount of radioactivity inhaled, absorbed, and exhaled in expired air while the animals were in the exposure chamber was not measured. Since both the plasma half-life, and the time for plasma concentrations to reach equilibrium following the onset of inhalation exposure, for HFC134a are on the order of 5 to 15 minutes (Riley *et al.*, 1979; Finch *et al.*, 1995),

measurement of radioactivity excreted post-exposure is reflective of HFC134a absorbed in the previous 5 to 15 minutes of exposure. As a result, the 1% absorption cited by Ellis *et al.* (1993) is a very minimum estimate, with actual absorption likely 4- to 10-fold higher (*i.e.*, the amount amounts recovered post-exposure should be more appropriately be divided by the amount inhaled over the last 5 to 15 minutes rather than the entire 60 minutes). Since HFC134a does not bioaccumulate following prolonged inhalation exposure, once the equilibrium concentration (plasma:air) has been reached, the amount of radioactivity recoverable post-exposure remains constant regardless of the exposure duration.

The highly conservative estimate of the inhalation absorption of HFC134a (*i.e.* 1%) cited by Ellis *et al.* (1993) can be used to calculate oral bioequivalent exposures for the 2-year chronic/carcinogenicity study from which the NOAEL of 10,000 ppm was established (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995). In this study, groups of Wistar-derived rats were exposed to HFC134a by inhalation at concentrations of 0, 2,500, 10,000 or 50,000 ppm for 6 hours/day, 5 days/week. This NOAEL of 10,000 ppm (42,500 mg/m^3) is associated with an external dose of approximately 18,973 mg/kg body weight based on the assumption that a Wistar-derived rat weighs approximately 400 grams and inhales about 0.37 m^3 over the course of a day (*i.e.*, 42,500 m^3/day x 6 hours/24 hours x 5 days/7 days / 0.400 kg body weight) (U.S. EPA, 1988).

Given the inhalation absorption rate of 1% cited by Ellis *et al.* (1993), the inhaled dose of approximately 18,973 mg/kg body weight/day would equate to an absorbed dose of approximately 190 mg/kg body weight/day (*i.e.*, 18,973 mg/kg body weight/day x 0.01). As a result, even if oral dosing was associated with 100% absorption, the NOAEL in the chronic inhalation study is, at a minimum, equivalent to a 190 mg/kg body weight/day oral dose. The equivalent oral dose is actually likely to be considerably greater since the inhalation absorption rate of 1% cited by Ellis *et al.* (1993), and used in the calculations, was based on the amount of radioactivity recovered post-exposure, not during the entire exposure period (Ellis *et al.*, 1993).

The NOAEL of 10,000 ppm established in the 2-year inhalation study in rats, equivalent to an oral exposure of at least 190 mg/kg body weight/day, can be compared to the estimated human exposure to HFC134a, under its intended conditions of use, to calculate a margin-of-safety. Given an oral bioequivalent NOAEL of at least 190 mg/kg body weight/day, there exists a more than a 430,000-fold margin of safety when compared to the estimated HFC134a intake of 0.00044 mg/kg body weight/day (*i.e.*, 190 mg/kg body weight/day / 0.00044 mg/kg body weight/day). These data provide assurance that human exposure to HFC134a under its intended conditions of use does not pose a safety concern.

LEYDIG CELL TUMORS

Administration of HFC134a at an inhalation concentration of 50,000 ppm, 6 hours/day, 5 days/week to groups of male Wistar rats for 2 years was associated with a statistically significant

increase in mean relative testes weight which correlated with an increased incidence of Leydig cell hyperplasia and benign Leydig cell tumors (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995). These effects were not observed at the lower exposure concentrations of 10,000 and 2,500 ppm. Also, there was no effect of treatment at any dose level on the incidence of Leydig cell hyperplasia or benign tumors in animals evaluated at 52 weeks.

In contrast to the results of the inhalation toxicity study (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995), the results of the chronic oral toxicity study (Longstaff *et al.*, 1984), in which Wistar-derived rats were treated with HFC134a by gavage at a dose of 300 mg/kg body weight/day for 52 weeks, followed by 73 weeks of observation, showed no adverse effect of treatment on the incidence of Leydig cell tumors. By comparison, in the same study, under the same experimental protocol, treatment with the closely related compound FC133a (1,1,1-trifluoro-2-chloroethane) was associated with a high incidence of Leydig cell tumors compared to controls (81% *versus* 15%). In addition, this compound was associated with uterine adenocarcinomas. As with HFC134a, FC133a is also non-mutagenic and non-genotoxic (Longstaff *et al.*, 1984).

In a 2-year inhalation study on HFC134a, an increased incidence of Leydig cell hyperplasia and benign tumours was reported in Wistar-derived rats. The process by which Leydig cell hyperplasia progresses to benign tumors is common in many strains of rats. As demonstrated by the oral toxicity study, the potency of this effect is much weaker for HFC134a than for FC133a (Longstaff *et al.*, 1984).

The exact mechanism by which Leydig cell tumors develop is not known (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995). However, it is likely to involve a hormonal response, especially in light of the finding of increased incidence of uterine adenocarcinomas in FC133a dosed rats (Longstaff *et al.*, 1984). Also, for both HFC143a and FC133a, the mechanism does not involve genotoxicity, and, therefore, would be associated with a threshold type dose-response relationship.

There are four important interspecies differences in the biology, physiology and pathology of Leydig cells that differentiate the rat from man. First, the spontaneous incidence of this tumor in the Wistar-derived rat strains is much higher, upwards of 100% (Boorman *et al.*, 1990), than that reported in humans. In most other rat strains, the spontaneous incidence is around 10%, while in humans the Leydig cell tumor incidence has been quoted to range from one-in-ten million to three-in-one million (Bär, 1992; Gilliland and Key, 1995). The high spontaneous incidence in Wistar-derived rat strains suggests a genetic predisposition of this strain to the development of these tumors. Second, the number of leutinizing hormone (LH) receptors present on individual Leydig cells has been reported to be 14-times greater in the rat compared to humans (Huhtaniemi, 1983). A high LH receptor concentration is likely to make the rat Leydig cell more susceptible to increases in LH levels (Prentice and Meikle, 1995). Third, in addition to quantitative differences in LH receptor concentration, there are qualitative differences in the types of receptors present on the Leydig cells of rats and humans. The rat Leydig cell

possesses luteinizing hormone releasing hormone (LHRH) receptors (Sharpe, 1988; Prentice and Meikle, 1995), while no LHRH receptors are found in the Leydig cells of humans (Clayton and Huhtaniemi, 1982), monkeys (Mann *et al.*, 1989) or mice (Wang *et al.*, 1983). Since LHRH has similar effects on the rat Leydig cell as LH (Sharpe, 1988; Prentice and Meikle, 1995), rat Leydig cells may be directly stimulated by hypothalamic and Sertoli cell release of LHRH, and by LHRH-induced release of LH from the pituitary. Fourth, the hormonal milieu in which the Leydig cells function is different in rats compared to man. In rats, serum concentrations of LH and testosterone decrease with advancing age (Chan *et al.*, 1977; Roberts *et al.*, 1989), while in man LH levels tend to increase with age (Rubens *et al.*, 1974).

Species- and strain- specificity of Leydig cell physiology likely plays a key role in the sensitivity of certain rat strains to Leydig cell tumor development. The lack of sensitivity of human Leydig cells is supported by the fact that several widely used pharmaceuticals, including cimetidine, finasteride, and isradipine (Alison *et al.*, 1994; McClain, 1994; Prentice and Meikle, 1995; Waalkes *et al.*, 1997), which induce increases in LH, and increase the incidence of Leydig cell tumors in rats, have shown no indication of testicular effects in humans (Roberts *et al.*, 1989; Bär, 1992, Crisp *et al.*, 1997).

Several studies have examined the mechanism(s) involved in the development of chemically induced Leydig cell tumors, particularly in rats (Rao *et al.*, 1992; Saez, 1994; Prentice and Meikle, 1995). Increases in Leydig cell tumors can be related to changes in the pituitary-testosterone feedback loop in many of the reported studies. Suggested mechanisms involve reduced secretion of testosterone from the Leydig cell (*i.e.*, from testicular damage), interference of testosterone utilization (*i.e.*, from inhibition of androgen binding), inhibition of the conversion of circulating testosterone to 5-α-dihydrotestosterone (*i.e.*, by inhibitors of 5-α-reductase), a potent controller of hypothalamic/pituitary LH release, or other changes which might result in the increased secretion of LH by the pituitary (Prentice and Meikle, 1995). Increased release of LH by the pituitary enhances Leydig cell proliferation, which, over an extended period, could result in hyperplasia/hypertrophy and the development of tumors (Prentice and Meikle, 1995).

Based on the species- and strain-specificity of occurrence, and on the known differences between humans and rats in the hypothalamic/pituitary/gonadal axis control of Leydig cell function, the increases in Leydig cell hyperplasia and in the incidence of benign Leydig cell tumors in the inhalation study with HFC134a are concluded to be of no relevance to humans exposed to trace quantities of HFC134a from its intended use as a food flavor extraction solvent. This conclusion is consistent with the conclusion of several reviews on the significance to humans of Leydig cell tumors in rats (Alison *et al.*, 1994; McClain, 1994; Prentice and Mielke, 1995).

TOXICITY OF METABOLITES

The only metabolite of HFC 134a isolated in both *in vitro* (Olson *et al.*, 1990a,b) and *in vivo* (Ellis *et al.*, 1993; Finch *et al.*, 1995) studies is trifluoroacetic acid. Ellis *et al.* (1993) reported that trifluoroacetic acid was the only metabolite identified in urine following exposure of male and female rats to 10,000 ppm HFC134a for one hour. The amount of trifluoroacetic acid excreted in urine and feces accounted for less than 0.15% of the inhaled dose. In a similar study, trifluoroacetylated proteins were not detected in F344 rats exposed to an atmospheric concentration of 10,000 ppm (42,500 mg/m^3) HFC134a for 6 hours, indicating that metabolism did not form radicals or other reactive intermediates, such as a trifluoroacetyl halide (Harris *et al.*, 1992).

HFC134a is not metabolized to monofluoroacetate, a known potent inhibitor of the Kreb's cycle (Gribble, 1973; Keller *et al.*, 1996). Monofluoroacetate is highly toxic with an oral LD_{50} value of 5.0 mg/kg body weight in albino rats (U.S. EPA IRIS, 2001). Similarly, fluorinated ethanes shown to be metabolized to monofluoroacetate have been reported to be acutely toxic (Keller *et al.*, 1996). In contrast, the metabolite of HFC134a, trifluoroacetate, is not known to inhibit ATP production and shows a much lower order of toxicity with an LD_{50} value of greater than 200 mg/kg body weight (Fraser and Kaminski, 1988).

In summary, the minimal amount of metabolism of HFC134a to trifluoroacetate does not pose a safety concern to humans exposed to trace quantities of HFC134a (*i.e.*, 0.00044 mg/kg body weight/day) under its intended conditions of use.

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Attachment 1

ATTACHMENT 1

ESTIMATED DAILY INTAKE OF ESSENTIAL OILS BY THE U.S. POPULATION FROM USE AS FOOD ADDITIVES

POUNDAGE SURVEY DATA

The Flavor & Extract Manufacturers' Association (FEMA) compiled survey data from 1995 submitted by 63 of its member companies and from data submitted by the National Association of Chewing Gum Manufacturers on behalf of its members. More than 2,400 flavoring substances used as food additives were included in the FEMA survey. Poundage data was not requested for the following:

a) flavoring substances exported or used in exported flavors or food products;

b) flavoring substances used in pharmaceutical or over-the-counter preparations;

c) flavoring substances used in oral hygiene products (*e.g.*, toothpaste and mouthwash);

d) flavoring substances used in tobacco products;

e) flavoring substances used in pet foods;

f) flavoring substances used in fragrances;

g) flavoring substances sold in 1995 to flavor companies, food manufacturers, or any other company;

h) flavoring substances used to make other discrete ingredients;

i) flavoring substances used for non-flavor related purposes

Poundage data on 215 essential oils was extracted from a CD-rom containing the survey results and imported into a spreadsheet. The data were then converted from poundage (pounds manufactured per year) to an estimate of daily *per capita* intake (mg/person/day) using the following calculation:

$$\text{Daily } \textit{per capita} \text{ intake} = \frac{\text{Poundage (lbs/yr)}}{2.2 \text{ lbs/kg} \times 260 \times 10^6 \text{ people} \times 365 \text{ days/yr}} \times 10^6 \text{ mg/kg}$$

The calculation was based on the assumption that a) 100% of the companies producing the essential oil have reported the poundage data; and b) 100% of the U.S. population is consuming the total poundage.

RESULTS

The total poundage and estimated daily *per capita* intake of essential oils from use as food additives are presented in Table 1 on a mg and mg per kilogram body weight basis.

Table 1 Total Poundage and Daily *Per Capita* Intake of Essential Oils

Total Poundage (lbs/yr)	Intake (mg/person/day)	Intake (mg/kg bw/day)
18,398,552.6	88.12	1.47

The poundage of the essential oils ranged from 0 for a number of oils, to 4,650,000 pounds per year for peppermint oil (*Mentha piperita L.*). A complete list of the essential oil poundage and *per capita* intake is presented in Appendix 1.

REFERENCES

Lucas, C.D.; Putnam, J.M.; Hallagan, J.B. 1999. 1995 Poundage and Technical Effects Update Survey. Flavor and Extract Manufacturers' Association (FEMA); Washington, DC.

Appendix 1

APPENDIX 1 POUNDAGE AND INTAKE DATA ON ESSENTIAL OILS

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8006-77-7	ALLSPICE OIL (PIMENTA OFFICINALIS LINDL.)	1600	7.66E-03	0.000127726
8013-76-1	ALMOND OIL, BITTER (FFPA) (PRUNUS SPP.)	13400	6.42E-02	0.001069707
8007-69-0	ALMOND OIL, SWEET	4	1.92E-05	3.19315E-07
8015-62-1	AMBRETTE ABSOLUTE OIL (HIBISCUS ABELMOSCHUS L.)	4	1.92E-05	3.19315E-07
8015-62-1	AMBRETTE SEED OIL (HIBISCUS ABELMOSCHUS L.)	4	1.92E-05	3.19315E-07
8015-65-4	AMYRIS, OIL (AMYRIS BALSAMIFERA L.)	130	6.23E-04	1.03778E-05
8015-64-3	ANGELICA ROOT OIL (ANGELICA ARCHANGELICA L.)	19	9.10E-05	1.51675E-06
8015-64-3	ANGELICA SEED OIL (ANGELICA ARCHANGELICA L.)	12	5.75E-05	9.57946E-07
8015-64-3	ANGELICA STEM OIL (ANGELICA ARCHANGELICA L.)	0	0.00E+00	0
8007-70-3	ANISE OIL (PIMPINELLA ANISUM L.)	13300	6.37E-02	0.001061724
8007-70-3	ANISE, STAR, OIL (ILLICIUM VERUM HOOK, F.)	12000	5.75E-02	0.000957946
72869-69-3	APRICOT KERNEL OIL (PRUNUS ARMENIACA L.)	43100	2.06E-01	0.003440623
2593321	ASAFOETIDA OIL (FERULA ASAFOETIDA L.)	170	8.14E-04	1.35709E-05
8014-71-9	BALM OIL (MELISSA OFFICINALIS L.)	39	1.87E-04	3.11333E-06
8016-42-0	BALSAM FIR OIL (ABIES BALSAMEA (L.) MILL.)	53	2.54E-04	4.23093E-06
8007-00-9	BALSAM OIL, PERU (MYROXYLON PEREIRAE KLOTZSCH)	4440	2.13E-02	0.00035444

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8015-73-4	BASIL OIL (OCIMUM BASILICUM L.)	2790	1.34E-02	0.000222722
	BAY LEAVES, W. INDIAN, OIL (PIMENTA RACEMOSA MILL)	140	6.71E-04	1.1176E-05
91721-75-4	BAY LEAVES, WEST INDIAN, OIL (PIMENTA ACRIS KOSTEL)	2210	1.06E-02	0.000176422
8007-48-5	BAY OIL, SWEET (LAURUS NOBILIS L.)	2470	1.18E-02	0.000197177
8007-75-8	BERGAMOT OIL (CITRUS AURANTIUM L. SUBSP. BERGAMIA WRIGHT ET ARN.)	16600	7.95E-02	0.001325159
8001-88-5	BIRCH TAR, OIL (BETULA PENDULA ROTH AND RELATED BETULA SPP.)	17	8.14E-05	1.35709E-06
68917-50-0	BIRCH, SWEET, OIL (BETULA LENTA L.)	4150	1.99E-02	0.00033129
8015-77-8	BOIS DE ROSE OIL (ANIBA ROSAEODORA DUCKE)	24600	1.18E-01	0.00196379
8016-94-2	BROMINATED VEGETABLE OIL	89800	4.30E-01	0.00716863
68650-46-4	BUCHU LEAVES OIL (BAROSMA SPP.)	570	2.73E-03	4.55024E-05
	BUTTER OIL	128000	6.13E-01	0.010218092
8008-98-8	CAJEPUT OIL (MELALEUCA LEUCADENDRON L.)	5	2.39E-05	3.99144E-07
8008-51-3	CAMPHOR, JAPANESE, WHITE, OIL (CINNAMOMUM CAMPHORA (L.) NEES ET EBERM.)	1220	5.84E-03	9.73912E-05
68606-83-7	CANANGA OIL (CANANGA ODORATA HOOK. F. AND THOMS.)	750	3.59E-03	5.98716E-05
8000-42-8	CARAWAY OIL (CARUM CARVI L.)	1840	8.81E-03	0.000146885
8000-66-6	CARDAMOM SEED OIL (ELLETARIA CARDAMOMUM (L.) MATON)	2870	1.37E-02	0.000229109
8015-88-1	CARROT OIL (DAUCUS CAROTA L.)	740	3.54E-03	5.90733E-05

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
2230693	CASCARILLA BARK OIL (CROTON SPP.)	4	1.92E-05	3.19315E-07
8007-80-5	CASSIA BARK OIL (CINNAMOMUM CASSIA BLUME)	112000	5.36E-01	0.008940831
8001-79-4	CASTOR OIL (RICINUS COMMUNIS L.)	33800	1.62E-01	0.002698215
8007-20-3	CEDAR LEAF OIL (THUJA OCCIDENTALIS L.)	22	1.05E-04	1.75623E-06
8000-27-9	CEDARWOOD OIL	150	7.18E-04	1.19743E-05
68603-22-5	CEDARWOOD OIL ALCOHOLS	3	1.44E-05	2.39487E-07
	CEDARWOOD OIL TERPENELESS	0.1	4.79E-07	7.98288E-09
68608-32-2	CEDARWOOD OIL TERPENES	44	2.11E-04	3.51247E-06
	CELERY LEAF OIL	60	2.87E-04	4.78973E-06
8015-90-5	CELERY SEED OIL (APIUM GRAVEOLENS L.)	1250	5.99E-03	9.97861E-05
8015-92-7	CHAMOMILE FLOWER, ENGLISH, OIL (ANTHEMIS NOBILIS L.)	450	2.16E-03	3.5923E-05
8002-66-2	CHAMOMILE FLOWER, HUNGARIAN, OIL (MATRICARIA CHAMOMILLA L.)	1850	8.86E-03	0.000147683
8015-92-7	CHAMOMILE FLOWER, ROMAN, OIL (ANTHEMIS NOBILIS L.)	800	3.83E-03	6.38631E-05
8000-44-0	CHERRY LAUREL OIL (FFPA) (PRUNUS LAUROCERASUS L.)	360	1.72E-03	2.87384E-05
	CILANTRO OIL	81	3.88E-04	6.46614E-06
8007-80-5	CINNAMON BARK OIL (CINNAMOMUM SPP.)	3640	1.74E-02	0.000290577
8007-80-5	CINNAMON LEAF OIL (CINNAMOMUM SPP.)	36900	1.77E-01	0.002945684
	CITRALLESS LEMON OIL	2340	1.12E-02	0.0001868
8000-29-1	CITRONELLA OIL (CYMBOPOGON NARDUS RENDLE)	6790	3.25E-02	0.000542038

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8016-63-5	CLARY OIL (SALVIA SCLAREA L.)	320	1.53E-03	2.55452E-05
8000-34-8	CLOVE BUD OIL (EUGENIA SPP.)	46500	2.23E-01	0.003712041
8000-34-8	CLOVE LEAF OIL (EUGENIA SPP.)	50900	2.44E-01	0.004063288
8000-34-8	CLOVE STEM, OIL (EUGENIA SPP.)	2560	1.23E-02	0.000204362
	COCONUT OIL	155000	7.42E-01	0.012373471
	COCONUT OIL DERIVED TRIGLYCERIDES	544000	2.61E+00	0.043426893
	COD LIVER OIL	1630	7.81E-03	0.000130121
	COFFEE OIL	1890	9.05E-03	0.000150877
8016-21-5	COGNAC OIL, GREEN	430	2.06E-03	3.43264E-05
8016-21-5	COGNAC OIL, WHITE	83	3.98E-04	6.62579E-06
8001-61-4	COPAIBA, OIL (SOUTH AMERICAN SPP. OF COPAIFERA L.)	57	2.73E-04	4.55024E-06
8008-52-4	CORIANDER OIL (CORIANDRUM SATIVUM L.)	6090	2.92E-02	0.000486158
	CORN OIL	394000	1.89E+00	0.031452566
68917-18-0	CORNMINT OIL	46600	2.23E-01	0.003720024
8023-88-9	COSTUS ROOT OIL (SAUSSUREA LAPPA CLARKE)	20	9.58E-05	1.59658E-06
8007-87-2	CUBEBS OIL (PIPER CUBEBA L. F.)	13	6.23E-05	1.03778E-06
8014-13-9	CUMIN OIL (CUMINUM CYMINUM L.)	3030	1.45E-02	0.000241881
94266-47-4	CURACAO PEEL OIL (CITRUS AURANTIUM L.)	100	4.79E-04	7.98288E-06
2233887	DAVANA OIL (ARTEMISIA PALLENS WALL.)	1520	7.28E-03	0.00012134

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8006-75-5	DILL OIL (ANETHUM GRAVEOLENS L.)	9020	4.32E-02	0.000720056
	DILL WEED OIL	69000	3.30E-01	0.00550819
8023-89-0	ELEMI OIL (CANARIUM SPP.)	150	7.18E-04	1.19743E-05
8007-27-0	ERIGERON OIL (ERIGERON CANDENSIS L.)	0	0.00E+00	0
8016-88-4	ESTRAGON OIL (ARTEMISIA DRACUNCULUS L.)	430	2.06E-03	3.43264E-05
8000-48-4	EUCALYPTUS OIL (EUCALYPTUS GLOBULUS LABILLE)	181000	8.67E-01	0.014449021
8006-84-6	FENNEL OIL, SWEET (FOENICULUM VULGARE MILL. VAR. DULCE DC.)	2600	1.25E-02	0.000207555
	FISH OIL	420	2.01E-03	3.35281E-05
8013-75-0	FUSEL OIL, REFINED	14400	6.90E-02	0.001149535
8023-91-4	GALANGAL ROOT OIL (ALPINIA SPP.)	0	0.00E+00	0
8023-91-4	GALBANUM OIL (FERULA SPP.)	100	4.79E-04	7.98288E-06
8000-78-0	GARLIC OIL (ALLIUM SATIVUM L.)	243000	1.16E+00	0.01939841
8000-46-2	GERANIUM ROSE OIL (PELARGONIUM GRAVEOLENS L'HER)	2440	1.17E-02	0.000194782
	GERANIUM, EAST INDIAN, OIL (CYMBOPOGON MARTINI STAPF.)	5	2.39E-05	3.99144E-07
8000-46-2	GERANIUM, OIL (PELARGONIUM SPP.)	61	2.92E-04	4.86956E-06
2230756	GINGER OIL (ZINGIBER OFFICINALE ROSC.)	8120	3.89E-02	0.00064821
	GRAPEFRUIT OIL CONC	3	1.44E-05	2.39487E-07
	GRAPEFRUIT OIL TERPENELESS	90	4.31E-04	7.1846E-06

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8016-20-4	GRAPEFRUIT OIL, EXPRESSED (CITRUS PARADISI MACF.)	196000	9.39E-01	0.015646454
8016-20-4	GRAPEFRUIT OIL, EXPRESSED (CITRUS PARADISI MACF.) (11X+ FOLD)	840	4.02E-03	6.70562E-05
8016-20-4	GRAPEFRUIT OIL, EXPRESSED (CITRUS PARADISI MACF.) (2X-5X FOLD)	9300	4.45E-02	0.000742408
8016-20-4	GRAPEFRUIT OIL, EXPRESSED (CITRUS PARADISI MACF.) (6X-10X FOLD)	2500	1.20E-02	0.000199572
8016-23-7	GUAIAC WOOD OIL (GUAIACUM SPP.)	420	2.01E-03	3.35281E-05
	HAY OIL	0.1	4.79E-07	7.98288E-09
2230630	HOPS OIL (HUMULUS LUPUS L.)	45	2.16E-04	3.5923E-06
	HORSERADISH, OIL (ARMORACIA LAPOTHIFOLIA GILIB.)	190	9.10E-04	1.51675E-05
8006-83-5	HYSSOP OIL (HYSSOPUS OFFICINALIS L.)	4	1.92E-05	3.19315E-07
8022-96-6	JASMINE OIL (JASMINUM GRANDIFLORUM L.)	18	8.62E-05	1.43692E-06
8012-91-7	JUNIPER OIL (JUNIPERUS COMMUNIS L.)	460	2.20E-03	3.67213E-05
8016-26-0	LABDANUM OIL (CISTUS SPP.)	18	8.62E-05	1.43692E-06
	LAUREL LEAVES OIL	250	1.20E-03	1.99572E-05
8022-15-9	LAVANDIN OIL (LAVANDULA HYDRIDA)	1	4.79E-06	7.98288E-08
8000-28-0	LAVENDER OIL (LAVANDULA OFFICINALIS CHAIX)	960	4.60E-03	7.66357E-05
	LEEK, OIL	13	6.23E-05	1.03778E-06
	LEMON ESSENCE OIL	57000	2.73E-01	0.004550244
8008-56-8	LEMON OIL (CITRUS LIMON (L.) BURM. F.)	2370000	1.14E+01	0.189194367

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8008-56-8	LEMON OIL (CITRUS LIMON (L.) BURM. F.) (2X-5X FOLD)	72600	3.48E-01	0.005795574
8008-56-8	LEMON OIL (CITRUS LIMON (L.) BURM. F.) (6X-10X FOLD)	7610	3.64E-02	0.000607498
68648-39-5	LEMON OIL TERPENELESS (CITRUS LIMON (L.) BURM. F.)	55300	2.65E-01	0.004414535
2230569	LEMONGRASS OIL (CYMBOPOGON CITRATUS DC. AND CYMBOPOGON FLEXUOSUS)	3240	1.55E-02	0.000258645
8008-26-2	LIME OIL (CITRUS AURANTIFOLIA (CHRISTMAN) SWINGLE)	713000	3.42E+00	0.056917968
8008-26-2	LIME OIL (CITRUS AURANTIFOLIA (CHRISTMAN) SWINGLE) (2X-5X FOLD)	4220	2.02E-02	0.000336878
	LIME OIL EXPRESSED	3	1.44E-05	2.39487E-07
	LIME OIL, DISTILLED	348000	1.67E+00	0.027780439
	LIME OIL, DISTILLED (2X-5X FOLD)	11300	5.41E-02	0.000902066
8008-26-2	LIME OIL, EXPRESSED	99600	4.77E-01	0.007950953
	LIME OIL, EXPRESSED (2X-5X FOLD)	8950	4.29E-02	0.000714468
68916-84-7	LIME OIL, TERPENELESS (CITRUS AURANTIFOLIA (CHRISTMAN) SWINGLE)	52200	2.50E-01	0.004167066
	LIME, ESSENCE OIL	380	1.82E-03	3.0335E-05
8006-86-8	LINALOE WOOD OIL (BURSERA DELPECHIANA POISS. AND OTHER BURSERA SPP.)	0	0.00E+00	0
	LIPOLIZED BUTTER OIL	62900	3.01E-01	0.005021234
8016-31-7	LOVAGE OIL (LEVISTICUM OFFICINALE KOCH)	49	2.35E-04	3.91161E-06
2230874	MACE OIL (MYRISTICA FRAGRANS HOUTT.)	840	4.02E-03	6.70562E-05
	MANDARIN ESSENCE OIL	0.1	4.79E-07	7.98288E-09

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8008-31-9	MANDARIN OIL, EXPRESSED	19600	9.39E-02	0.001564645
	MANDARIN OIL, EXPRESSED (11X+ FOLD)	80	3.83E-04	6.38631E-06
8008-31-9	MANDARIN OIL, EXPRESSED (6X-10X FOLD)	3	1.44E-05	2.39487E-07
	MANDARIN PETITGRAIN OIL TERPENELESS	330	1.58E-03	2.63435E-05
2233467	MARJORAM OIL, SWEET (ORIGANUM MAJORANA)	1350	6.47E-03	0.000107769
85085-26-3	MASSOIA BARK OIL (CRYPTOCARYA MASSOIO)	66	3.16E-04	5.2687E-06
	MINERAL OIL	47	2.25E-04	3.75196E-06
	MUSTARD OIL	96200	4.61E-01	0.007679535
8016-37-3	MYRRH OIL (COMMIPHORA SPP.)	19	9.10E-05	1.51675E-06
8016-38-4	NEROLI BIGARDE OIL (CITRUS AURANTIUM L.)	340	1.63E-03	2.71418E-05
8008-45-5	NUTMEG OIL (MYRISTICA FRAGRANS HOUTT.)	140000	6.71E-01	0.011176039
8016-36-2	OLIBANUM OIL (BOSWELLIA SPP.)	3	1.44E-05	2.39487E-07
8002-72-0	ONION OIL (ALLIUM CEPA L.)	4140	1.98E-02	0.000330491
8021-36-1	OPOPONAX, OIL	22	1.05E-04	1.75623E-06
	ORANGE AROMA OIL	49	2.35E-04	3.91161E-06
	ORANGE AROMA OIL (6X-10X FOLD)	1490	7.14E-03	0.000118945
	ORANGE ESSENCE OIL	157000	7.52E-01	0.012533129
	ORANGE ESSENCE OIL (2X-5X FOLD)	6040	2.89E-02	0.000482166
	ORANGE ESSENCE OIL (6X-10X FOLD)	7930	3.80E-02	0.000633043

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
	ORANGE ESSENSE OIL (11X+ FOLD)	59	2.83E-04	4.7099E-06
68606-94-0	ORANGE OIL DISTILLED (CITRUS SINENSIS (L.) OSBECK)	436000	2.09E+00	0.034805377
68606-94-0	ORANGE OIL DISTILLED (CITRUS SINENSIS (L.) OSBECK) (11X+ FOLD)	9300	4.45E-02	0.000742408
68606-94-0	ORANGE OIL DISTILLED (CITRUS SINENSIS (L.) OSBECK) (2X-5X FOLD)	67300	3.22E-01	0.005372481
68606-94-0	ORANGE OIL DISTILLED (CITRUS SINENSIS (L.) OSBECK) (6X-10X FOLD)	53000	2.54E-01	0.004230929
8008-57-9	ORANGE OIL TERPENELESS (CITRUS SINENSIS (L.) OSBECK)	61500	2.95E-01	0.004909474
68916-04-1	ORANGE PEEL OIL, BITTER (CITRUS AURANTIUM L.)	3680	1.76E-02	0.00029377
68916-04-1	ORANGE PEEL OIL, BITTER (CITRUS AURANTIUM L.) (2X-5X FOLD)	4310	2.06E-02	0.000344062
8008-57-9	ORANGE PEEL OIL, SWEET (CITRUS SINENSIS (L.) OSBECK)	1290000	6.18E+00	0.102979213
8008-57-9	ORANGE PEEL OIL, SWEET (CITRUS SINENSIS (L.) OSBECK) (11X-20X FOLD)	10300	4.93E-02	0.000822237
8008-57-9	ORANGE PEEL OIL, SWEET (CITRUS SINENSIS (L.) OSBECK) (2X-5X FOLD)	160000	7.66E-01	0.012772616
8008-57-9	ORANGE PEEL OIL, SWEET (CITRUS SINENSIS (L.) OSBECK) (6X-10X FOLD)	44000	2.11E-01	0.003512469
68606-94-0	ORANGE PEEL, SWEET, OIL, TERPENELESS (CITRUS SINENSIS L. OSBECK)	14300	6.85E-02	0.001141553
2230843	ORIGANUM OIL (EXTRACTIVE) (THYMUS CAPITATUS L. HOFFMANNS & LINK)	16900	8.09E-02	0.001349108
8002-73-1	ORRIS CONCRETE LIQUID OIL (IRIS FLORENTINA L.)	460	2.20E-03	3.67213E-05
8014-19-5	PALMAROSA OIL (CYMBOPOGON MARTINI (ROXB.) STAPF)	460	2.20E-03	3.67213E-05

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8000-68-8	PARSLEY OIL (PETROSELINUM SPP.)	890	4.26E-03	7.10477E-05
2233340	PATCHOULY OIL (POGOSTEMON SPP.)	450	2.16E-03	3.5923E-05
	PEANUT OIL	510000	2.44E+00	0.040712712
8013-99-8	PENNYROYAL OIL (MENTHA PULEGIUM L.)	220	1.05E-03	1.75623E-05
8006-82-4	PEPPER, BLACK, OIL (PIPER NIGRUM L.)	1060	5.08E-03	8.46186E-05
8006-82-4	PEPPER, WHITE, OIL (PIPER NIGRUM L.)	130	6.23E-04	1.03778E-05
8006-90-4	PEPPERMINT OIL (MENTHA PIPERITA L.)	4650000	2.23E+01	0.371204138
	PEPPERMINT OIL TERPENELESS	56900	2.73E-01	0.004542261
8014-17-3	PETITGRAIN OIL (CITRUS AURANTIUM L.)	7330	3.51E-02	0.000585145
	PETITGRAIN OIL TERPENELESS	150	7.18E-04	1.19743E-05
8008-56-8	PETITGRAIN, LEMON, OIL (CITRUS LIMON (L.) BURM. F.)	490	2.35E-03	3.91161E-05
8014-17-3	PETITGRAIN, MANDARIN OIL (CITRUS RETICULATA BLANCO VAR. MANDARIN)	17900	8.57E-02	0.001428936
8006-77-7	PIMENTA LEAF OIL (PIMENTA OFFICINALIS LINDL.)	7290	3.49E-02	0.000581952
	PINE BARK, WHITE, OIL (PINUS STROBUS L.)	1	4.79E-06	7.98288E-08
8021-29-2	PINE NEEDLE OIL (ABIES SPP.)	230	1.10E-03	1.83606E-05
8000-26-8	PINE NEEDLE, DWARF, OIL (PINUS MUGO TURRA VAR. PUMILIO (HAENKE) ZENARI)	33	1.58E-04	2.63435E-06
8023-99-2	PINE SCOTCH OIL (PINUS SYLVESTRIS L.)	140	6.71E-04	1.1176E-05
97435-14-8	PINE TAR OIL (PINUS PALUSTRIS MILL. AND OTHER PINUS SPP.)	0	0.00E+00	0

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
	PINE, WHITE, OIL (PINUS SPP.)	41	1.96E-04	3.27298E-06
8007-01-0	ROSE OIL, TRUE OTTO, BULGARIAN, (ROSA DAMASCENA MILL.)	150	7.18E-04	1.19743E-05
8000-25-7	ROSEMARY OIL (ROSEMARINUS OFFICINALIS L.)	3230	1.55E-02	0.000257847
8014-29-7	RUE OIL (RUTA GRAVEOLENS L.)	6	2.87E-05	4.78973E-07
	RUM FUSEL OIL	20	9.58E-05	1.59658E-06
8022-56-8	SAGE OIL (SALVIA OFFICINALIS L.)	1710	8.19E-03	0.000136507
8022-56-8	SAGE OIL, SPANISH (SALVIA LAVANDULAEFOLIA VAHL.)	650	3.11E-03	5.18888E-05
8006-87-9	SANDALWOOD OIL, YELLOW (SANTALUM ALBUM L.)	98	4.69E-04	7.82323E-06
8016-68-0	SAVORY SUMMER OIL (SATUREJA HORTENSIS L.)	27	1.29E-04	2.15538E-06
8016-68-0	SAVORY WINTER OIL (SATUREJA MONTANA L.)	0	0.00E+00	0
68917-52-2	SCHINUS MOLLE OIL (SCHINUS MOLLE L.)	2780	1.33E-02	0.000221924
	SESAME OIL	25000	1.20E-01	0.001995721
8016-69-1	SNAKEROOT OIL, CANADIAN (ASARUM CANADENSE L.)	11	5.27E-05	8.78117E-07
	SOYBEAN OIL, PART HYDROG	195000	9.34E-01	0.015566625
8008-79-5	SPEARMINT OIL (MENTHA SPICATA L.)	1570000	7.52E+00	0.12533129
	SPEARMINT OIL TERPENELESS	14100	6.75E-02	0.001125587
8016-78-2	SPIKE LAVENDER OIL (LAVANDULA SPP.)	0.3	1.44E-06	2.39487E-08
8008-80-8	SPRUCE OIL (TSUGA AND PICEA SPP.)	150	7.18E-04	1.19743E-05
	STYRAX OIL	2	9.58E-06	1.59658E-07

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
8016-84-0	TAGETES OIL (TAGETES ERECTA L.; T. PATULA L.; OR T. GLANDULIFERA SCHRANK)	220	1.05E-03	1.75623E-05
72869-73-9	TANGELO OIL	630	3.02E-03	5.02922E-05
8008-31-9	TANGERINE OIL (CITRUS RETICULATA BLANCO)	59800	2.86E-01	0.004773765
8008-31-9	TANGERINE OIL (CITRUS RETICULATA BLANCO) (11X+ FOLD)	8	3.83E-05	6.38631E-07
8008-31-9	TANGERINE OIL (CITRUS RETICULATA BLANCO) (2X-5X FOLD)	6870	3.29E-02	0.000548424
8008-31-9	TANGERINE OIL (CITRUS RETICULATA BLANCO) (6X-10X FOLD)	25	1.20E-04	1.99572E-06
68647-73-4	TEA TREE OIL	63	3.02E-04	5.02922E-06
8007-46-3	THYME OIL(THYMUS VULGARIS L.)	2340	1.12E-02	0.0001868
8007-46-3	THYME, WHITE, OIL (THYMUS VULGARIS L.)	100	4.79E-04	7.98288E-06
2236870	TUBEROSE OIL (POLIANTHES TUBEROSA L.)	8	3.83E-05	6.38631E-07
8008-88-6	VALERIAN ROOT OIL (VALERIANA OFFICINALIS L.)	20	9.58E-05	1.59658E-06
8008-89-7	VEGETABLE OIL	2220000	1.06E+01	0.17722004
8016-96-4	VETIVER, OIL (VETIVERIA ZIZANIOIDES STAPF)	90	4.31E-04	7.1846E-06
	WHEAT GERM OIL	1	4.79E-06	7.98288E-08
	WINE FUSEL OIL	1450	6.95E-03	0.000115752
68917-75-9	WINTERGREEN OIL (GAULTHERIA PROCUMBENS L.)	2960	1.42E-02	0.000236293
8008-93-3	WORMWOOD OIL (ARTEMISIA ABSINTHIUM L.)	19	9.10E-05	1.51675E-06
8006-81-3	YLANG YLANG OIL (CANANGA ODORATA HOOK. F. AND THOMAS)	97	4.65E-04	7.7434E-06

CAS #	ESSENTIAL OIL	FEMA POUNDAGE (lbs/year)	INTAKE (mg/person/day)	INTAKE (mg/kg bw/day)
TOTAL		18398552.6	88.1241	1.46873524

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SUPPLEMENT TO GRAS NOTIFICATION FOR HFC134a

(GRASN 82)

January 25, 2002

CANTOX

INTRODUCTION

- Qualified experts concluded that the available data supported the conclusion that HFC134a (1,1,1-2-tetrafluoroethane) was GRAS for use as an extraction solvent for food flavoring substances
- Subsequently, representatives of FDA requested clarification of several issues pertaining to the safety evaluation of HFC134a as an extraction solvent for flavors

CANTOX

INTRODUCTION cont'd

- **Issues to be addressed included:**
  - provision of a more refined estimate of human exposure
  - expansion of the rationale for the use of inhalation data to assess oral safety
  - evaluation of the significance to humans of Leydig cell tumors in rats
  - a discussion of the potential for metabolism to fluoroacetate, an inhibitor of the Kreb's cycle

CANTOX

ESTIMATE OF HUMAN EXPSOURE

- **In the original GRAS notification, human exposure was estimated to range from 0.05 to 0.5 mg/kg bw/day based on:**
  - a 60 kg person consuming 3,000 grams of food per day (U.S. FDA, 1999)
  - the assumption that flavoring substances constitute from 0.1 to 1.0% of the diet (*i.e.*, consumption of 3 to 30 grams of flavors/day)
  - HFC134a would be present in the flavor extracts at the maximum concentration of 1,000 ppm

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ESTIMATE OF HUMAN EXPSOURE cont'd

- **The exposure estimate was refined by:**
  - Assuming that all essential oils in the FEMA poundage survey are extracted with HFC134a
  - incorporation of the results of analytical studies showing that HFC134a residues in extracted flavors will typically not exceed 300 ppm (w/w)

CANTOX

ESTIMATE OF HUMAN EXPOSURE

FEMA Poundage Data

- **Poundage (usage) data on 215 essential oils was extracted from a CD-rom containing the survey results and imported to a spreadsheet. The data were then converted from poundage (pounds manufactured per year) to an estimate of daily *per capita* intake (mg/person/day) using the following calculation:**

$$\text{Daily } \textit{per capita} \text{ intake (mg/person/day)} = \frac{\text{Poundage (lbs/yr)} \times 10^{6} \text{ mg/kg}}{2.2 \text{ lbs/kg} \times 260 \times 10^{6} \text{ people} \times 365 \text{ days/yr}}$$

CANTOX

000249

ESTIMATE OF HUMAN EXPOSURE

FEMA Poundage Data

- **The calculation was based on the assumption that 100% of the U.S. population is consuming the total poundage.**

Total Poundage (lbs/yr),	Intake (mg/person/day)	Intake (mg/kg bw/day)
18, 398, 552	88.12	1.47

- **The human intake of essential oils, assuming that consumption was evenly distributed throughout the population, was calculated to be 1.47 mg/kg body weight/day**
- **The calculated intake of essential oils potentially used for food flavoring was from 30- to 300-fold lower than the previous estimate**

CANTOX

ESTIMATE OF HUMAN EXPOSURE

Residue Data

- **Originally, all flavoring substances were assumed to contain 1,000 ppm HFC134a, the maximum amount indicated in the product specifications**
- **Results of analytical studies conducted by Ineos Fluor, Ltd., demonstrate that the actual amount of residual solvent remaining in extracted oils is typically less than 300 ppm**

CANTOX

000250

ESTIMATE OF HUMAN EXPOSURE

Residue Data

Extract	Solvent Removal Conditions	Residual HFC134a (ppm)
Magnolia bark	Nitrogen purge at 45 °C	348
Magnolia bark	Nitrogen purge at 60 °C	65
Star anise	Nitrogen purge at ambient temperature	Not detected
Star anise	evacuation at 50 °C	236
Clove	Nitrogen purge at ambient temperature	103
Clove	evacuation at 50 °C	188
Ginger	evacuation at 40 °C	177
Orange	Nitrogen purge at ambient temperature	101
Vanilla	Nitrogen purge at 50 °C	107
Juniper oil	Evacuate at ambient temperature	102

CANTOX

ESTIMATE OF HUMAN EXPOSURE

Summary

- **Human exposure to HFC134a under its intended conditions of use is a product of the amount of HFC134a-extracted substances consumed and the concentration remaining in the extracted substances**
- **Amount of extracted oils consumed was calculated to be 1.47 mg/kg bw/day**
- **Residual HFC134a concentration was established to be at a maximum of 300 ppm**

- Human exposure = amount of extracted oils consumed x concentration of residual HFC134a

CANTOX

ESTIMATE OF HUMAN EXPOSURE cont'd

- Human exposure to HFC134a was estimated to be 0.00044 mg/kg body weight/day (*i.e.*, 1.47 mg/kg body weight/day x 300/1,000,000)
- This value does not account for volatilization (b.p. of –26 °C) or loss during food processing

CANTOX

USE OF INHALATION DATA TO ESTIMATE ORAL SAFETY

- The results of peer-reviewed inhalation studies (Hodge *et al.*, 1980; Lu, 1981; Hext, 1989; Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995; Alexander *et al.*, 1996) were used as the primary source of data to characterize toxicity
- Inhalation data were supported by a single 52-week oral toxicity study (Longstaff *et al.*, 1984)
- Since systemic toxicity is related to blood concentrations and AUC, inhalation studies providing for systemic exposure can be used to qualitatively characterize systemic toxicity

CANTOX

USE OF INHALATION DATA TO ESTIMATE ORAL SAFETY cont'd

- **For HFC134a, pharmacokinetic studies (Riley *et al.*, 1979; Finch *et al.*, 1995; Alexander *et al.*, 1996) show that inhalation exposure of rats to 10,000 ppm produces blood concentrations of HFC134a in the range of 10 to 20 mg/L (Riley *et al.*, 1979).**
- **Given that no systemic toxicity was observed in the chronic studies at 10,000 ppm, exposures to HFC134a producing blood concentrations in the range of 10 to 20 mg/L, whether achieved by inhalation or through oral dosing, are not associated with systemic toxicity**

CANTOX

USE OF INHALATION DATA TO ESTIMATE ORAL SAFETY

- **Quantitative estimates of oral toxicity can be established from inhalation data through route-to-route extrapolation that accounts for variances in absorption rates by different routes of exposure (U.S., EPA, 1987; Gerrity and Henry, 1990; Dourson and Felter, 1997)**
- **This technique has been used by the U.S. EPA to establish oral reference doses (RfD) on the basis of inhalation studies when no adequate oral studies existed**
- **The amount of HFC143a absorbed through inhalation exposure has been cited to be 1% (Ellis *et al.*, 1993)**
  - the 1% absorption cited by Ellis *et al.* (1993) is a lower bound estimate since these researchers measured only radioactivity recovered post-exposure, not radioactivity absorbed and excreted while the animals were in the exposure chamber

CANTOX

USE OF INHALATION DATA TO ESTIMATE ORAL SAFETY

- The conservative estimate of the inhalation absorption of HFC134a (*i.e.* 1%) can be used to calculate oral bioequivalent exposures for the 2-year chronic/carcinogenicity study from which the NOAEL of 10,000 ppm was established (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995)
- The NOAEL of 10,000 ppm (42,500 mg/m^3) is associated with an external dose of approximately 18,973 mg/kg body weight based on the assumption that a rat weighs and breathes 400 grams and 0.37 m^3 of air, respectively (*i.e.*, 42,500 m^3/day x 6 hours/24 hours x 0.37 m^3 x 5 days/7 days / 0.400 kg body weight)
- Based on a minimum of 1% absorption, the external inhaled dose of approximately 18,973 mg/kg body weight/day would equate to an absorbed dose of approximately 190 mg/kg body weight/day (*i.e.*, 18,973 mg/kg body weight/day x 0.01)

CANTOX

USE OF INHALATION DATA TO ESTIMATE ORAL SAFETY

- The oral bioequivalent dose of 190 mg/kg bw/day dose established as the NOAEL in the chronic inhalation study provides for a more than a 430,000-fold margin of safety when compared to the estimated HFC134a intake of 0.00044 mg/kg body weight/day (*i.e.*, 190 mg/kg body weight/day / 0.00044 mg/kg body weight/day)
- The preceding analysis provides assurance that human exposure to HFC134a under its intended conditions of use does not pose a safety concern

CANTOX

LEYDIG CELL TUMORS

- **In the chronic inhalation study, administration of HFC134a at a concentration of 50,000 ppm, 6 hours/day, 5 days/week to groups of male Wistar rats was associated with an increased incidence of Leydig cell hyperplasia and benign Leydig cell tumors (Hext and Parr-Dobrzanski, 1993; Collins *et al.*, 1995)**
- **No effects on the testes were observed at the lower exposure concentrations of 10,000 and 2,500 ppm**
- **The chronic oral toxicity study (Longstaff *et al.*, 1984), in which rats were treated with HFC134a at a dose of 300 mg/kg body weight/day for 52 weeks, showed no adverse effect of treatment on the incidence of Leydig cell tumors**
  - in the oral study, another related compound FC133a (1,1,1-trifluoro-2-chloroethane) was associated with a high incidence of Leydig cell tumors

CANTOX

LEYDIG CELL TUMORS

Significance to humans

- **The exact mechanism by which Leydig cell tumors develop is not known, but likely involves a hormonal response through LH**
- **There are three important interspecies differences in the biology, physiology and pathology of Leydig cells that differentiate the rat from man**
  - spontaneous incidence of Leydig cell tumors in the Wistar-derived rat strains is much higher than that reported in humans
  - the number of leutinizing hormone (LH) receptors present on individual Leydig cells has been reported to be 14-times greater in the rat compared to humans (Huhtaniemi, 1983)

CANTOX

LEYDIG CELL TUMORS cont'd

- the hormonal milieu in which the Leydig cells function is different in rats compared to man [*e.g.*, in rats, serum concentrations of LH and testosterone decrease with advancing age (Chan *et al.*, 1977; Roberts *et al.*, 1989), while in man LH levels tend to increase with age (Rubens *et al.*, 1974)]

CANTOX

LEYDIG CELL TUMORS

Significance to humans

- **Species- and strain- specificity of Leydig cell physiology likely plays a key role in the sensitivity of certain rat strains to Leydig cell tumor development**
- **The lack of sensitivity of human Leydig cells is supported by the fact that several widely used pharmaceuticals, including cimetidine, finasteride, and isradipine (Alison *et al.*, 1994; McClain, 1994; Prentice and Meikle, 1995; Waalkes *et al.*, 1997), which induce increases in LH, and increase the incidence of Leydig cell tumors in rats, have shown no indication of testicular effects in humans (Roberts *et al.*, 1989; Bär, 1992, Crisp *et al.*, 1997)**

CANTOX

LEYDIG CELL TUMORS cont'd

- **Based on the species- and strain-specificity of the occurrence of Leydig cell tumors, and on key species differences in Leydig cell physiology, the increases in benign Leydig cell tumors in the inhalation study with HFC134a are concluded to be of no relevance to humans exposed to trace quantities of HFC134a from its intended use as a food flavor extraction solvent**
  - several prominent researchers have concluded that the finding of Leydig cell tumors in rats is often of no relevance to humans (Alison *et al.*, 1994; McClain, 1994; Prentice and Mielke, 1995)

CANTOX

TOXICITY OF METABOLITES

- **The only metabolite of HFC134a identified *in vitro* and *in vivo* is trifluoroacetic acid**
- **Trifluoroacetic acid is a very minor metabolite, accounting for less than 0.15% of the inhaled dose (measured as radioactivity)**
- **Trifluoroacetylated proteins were not detected in F344 rats exposed to an HFC134a at 10,000 for 6 hours, indicating that metabolism did not form radicals or other reactive intermediates (Harris *et al.*, 1992)**
- **HFC134a is not metabolized to monofluoroacetate, a known potent inhibitor of the Kreb's cycle (Gribble, 1973; Keller *et al.*, 1996)**
- **Trifluoroacetate has an oral LD_{50} value of greater than 200 mg/kg body weight (Fraser and Kaminski, 1988)**
- **Metabolism of HFC134a does not pose a safety concern to humans**

CANTOX

CONCLUSIONS

- Using the FEMA poundage survey, and analytical data provided by Ineos Fluor, Ltd., human exposure to HFC134a was estimated to be 0.00044 mg/kg bw/day; more than 100- to 1,000-fold lower than the previous estimate
- Through route-to-route extrapolation, and incorporation of the inhalation absorption value of 1% cited by Ellis *et al.* (1993), the oral bioequivalent dose associated with the NOAEL of 10,000 ppm in the chronic inhalation study was calculated to be 190 mg/kg bw/day
- Oral bioequivalent NOAEL of 190 mg/kg bw/day provides for more than a 430,000-fold margin of safety when compared to the estimated HFC134 intake

CANTOX

CONCLUSIONS cont'd

- Due to significant species- and strain-specificity, the increase in the incidence of Leydig cell tumors associated with the highest concentration tested (50,000 ppm) in the chronic inhalation study are of no relevance to humans
- HFC134a is metabolized to trifluoroacetic acid, not monofluoroacetate, and does not pose a safety concern
- HFC134a is GRAS

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AM

Dietz, Jason

From:
Sent: Monday, February 11, 2002 12:01 PM
To: Dietz, Jason
Cc:
Subject: GRAS Notice No. GRN 000082

Dear Jason:

Dr. Gareth Robinson at Ineos Fluor, Ltd. has confirmed that the specification for residual HFC 134a in extracted food flavors and flavorings will be reduced from 1000 ppm to 300 ppm. The 300 ppm level is fully consistent with the analytical data presented during our recent meeting.

If you need confirmation of this specification change in a letter, just let me know and I will fax one to you. Please give me a call when you have a moment to discuss where we go from here.

Thank you,

Diane McColl
Counsel to Ineos Fluor Ltd.

Diane B. McColl
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700 Thirteenth St. N.W.
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000326

AM

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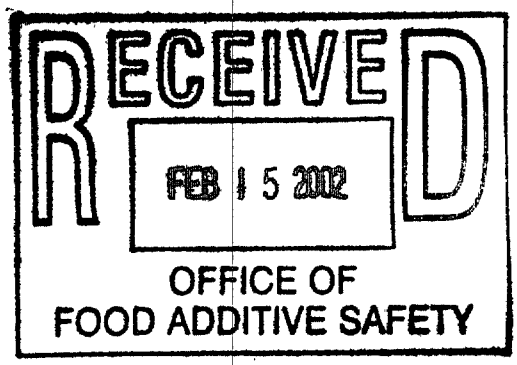
*NOT ADMITTED IN DC

February 12, 2002



BY FEDERAL EXPRESS

Jason Dietz
Division of Biotech & GRAS Notice Review
Office of Food Additive Safety
FDA Center for Food Safety and Applied Nutrition
Harvey W. Wiley Federal Building (CPK1)
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Supplement to GRAS Notice (GRN 82) for use of 1,1,1,2-tetrafluoroethane (HFC 134a) as an extraction solvent

Dear Mr. Dietz:

As recommended, Ineos Fluor, Ltd. has reduced its specifications for HFC 134a residues in extracted food flavors and flavorings from ≤1,000 ppm to ≤300 ppm. All other specifications remain as stated in GRAS Notice.

If you have any further questions concerning this matter, please do not hesitate to contact me.

Sincerely,

Diane B. McColl
Counsel to Ineos Fluor, Ltd.

DBM/dmh

000330

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Reference List for Industry Submission, GRN 000082

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
0000047 - 000053	European Commission Directorate-General on Industry Scientific Committee For Food	Opinion on the Safety in Use of 1,1,1,2-tetrafluoroethane as a Solvent for Flavour Extraction	December 1995	Reports of the Scientific Committee for Food	38th Series, Number 38
000054 - 000073	ECETOC	1,1,1 2-Tetrafluoroethane (HFC-134a) CAS No. 811-97-2: Absorption, Distribution, Metabolism and Elimination	February 1995	Joint Assessment of Commodity Chemicals	Number 31, Section 7, pgs 14-29
000111 - 000198	U.S. EPA	Risk Assessment Guidelines of 1986	1987	EPA	NA
000199 - 000207	Dourson, Michael L.; Felter, Susan P.	Route-to-Route Extrapolation of the Toxic Potency of MTBE	1997	Risk Analysis	Volume 17, Number 6, pgs 717-725
000208 - 000216	Alison, Roger H.; Capen, Charles C.; Prentice, David E.	Neoplastic Lesions of Questionable Significance to Humans	March-April 1994	Toxicologic Pathology	Volume 22, Number 2, pgs 179-186
000217 - 000234	McClain, R. Michael	Mechanistic Considerations in the Regulation and Classification of Chemical Carcinogens	1994	Nutritional Toxicology	Chapter 14, pgs 273-304
000235 - 000246	Prentice, D. E.; Meikle, A. W.	A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparisons with man	July 1995	Human and Experimental Toxicology	Volume 14, Number 7, pgs 562-572
000259 - 000267	Dourson, Michael L.; Felter, Susan P.	Route-to-Route Extrapolation of the Toxic Potency of MTBE	1997	Risk Analysis	Volume 17, Number 6, pgs 717-725
000268 - 000276	Alison, Roger, H.; Capen, Charles C.; Prentice, David E.	Neoplastic Lesions of Questionable Significance to Humans	March-April 1994	Toxicologic Pathology	Volume 22, Number 2, pgs 179-186
000277 - 000294	McClain, R. Michael	Mechanistic Considerations in the Regulation and Classification of Chemical Carcinogens	1994	Nutritional Toxicology	Chapter 14, pgs 273-304

NA- Not applicable

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000295 - 000306	Prentice, D. E.; Meikle, A. W.	A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparisons with man	July 1995	Human & Experimental Toxicology	Volume 14, Number 7, pgs 562-572
000307 - 000325	Bar, Albert	Significance of Leydig Cell Neoplasia in Rats Fed Lactitol or Lactose	1992	Journal of the American College of Toxicology	Volume 11, Number 2, pgs 189-207

NA- Not applicable